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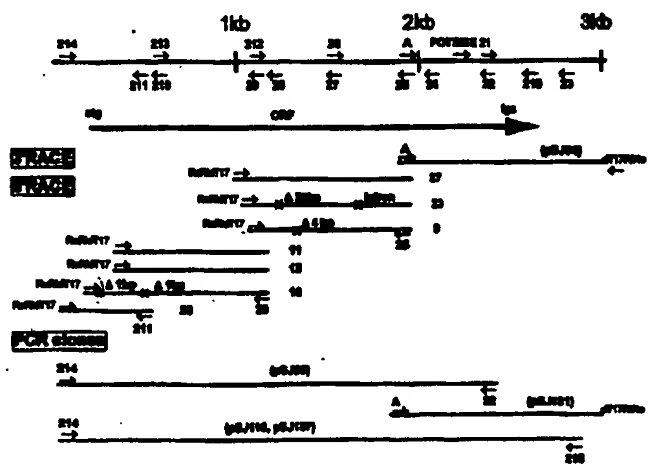

(57) Abstract

Disclosed is a nucleic acid sequence encoding a polypeptide having starch branching enzyme (SBE) activity, the encoded polypeptide comprising an effective portion of the amino acid sequence shown in Figure 4 or Figure 13.

Title: **Improvements in or Relating to Starch Content of Plants**

Field of the Invention

This invention relates to novel nucleic acid sequences, vectors and host cells comprising the nucleic acid sequence(s), to polypeptides encoded thereby, and to a method of altering a host cell by introducing the nucleic acid sequence(s) of the invention.

Background to the Invention

Starch consists of two main polysaccharides, amylose and amylopectin. Amylose is a linear polymer containing α-1,4 linked glucose units, while amylopectin is a highly branched polymer consisting of a α-1,4 linked glucan backbone with α-1,6 linked glucan branches. In most plant storage reserves amylopectin consitutes about 75% of the starch content. Amylopectin is synthesized by the concerted action of soluble starch synthase and starch branching enzyme [α-1,4 glucan: α-1,4 glucan 6-glycosyltransferase, EC 2.4.1.18]. Starch branching enzyme (SBE) hydrolyses α-1,4 linkages and rejoins the cleaved glucan, via an α-1,6 linkage, to an acceptor chain to produce a branched structure. The physical properties of starch are strongly affected by the relative abundance of amylose and amylopectin, and SBE is therefore a crucial enzyme in determining both the quantity and quality of starches produced in plant systems.

Starches are commercially available from several plant sources including maize, potato and cassava. Each of these starches has unique physical characteristics and properties and a variety of possible industrial uses. In maize there are a number of naturally occurring mutants which have altered starch composition such as high amylopectin types ("waxy" starches) or high amylose starches but in potato and cassava no such mutants exist on a commercial basis as yet.

Genetic modification offers the possibility of obtaining new starches which may have novel and potentially useful characteristics. Most of the work to date has involved potato plants because they are amenable to genetic manipulation i.e. they can be transformed using Agrobacterium and regenerated easily from tissue culture. In addition many of the genes involved in starch biosynthesis have been cloned from potato and thus are available as targets for genetic manipulation, for example, by antisense inhibition of expression or sense suppression.

Cassava (*Manihot esculenta* L. Crantz) is an important crop in the tropics, where its starch-filled roots are used both as a food source and increasingly as a source of starch. Cassava is a high yielding perennial crop that can grow on poor soils and is also tolerant of drought. Cassava starch being a root-derived starch has properties similar but not identical to potato starch and is composed of 20-25% amylose and 75-80% amylopectin (Rickard *et al.*, 1991. Trop. Sci. 31, 189-207). Some of the genes involved in starch biosynthesis have been cloned from cassava, including starch branching enzyme I (SBE I) (Salehuzzaman *et al.*, 1994 Plant Science 98, 53-62), and granule bound starch synthase I (GBSS I) (Salehuzzaman *et al.*, 1993 Plant Molecular Biology 23, 947-962) and some work has been done on their expression patterns although only in *in vitro* grown plants (Salehuzzaman *et al.*, 1994 Plant Science 98, 53-62).

In most plants studied to date e.g. maize (Boyer & Preiss, 1978 Biochem. Biophys. Res. Comm. *80*, 169-175), rice (Smyth, 1988 Plant Sci. *57*, 1-8) and pea (Smith, Planta *175*, 270-279), two forms of SBE have been identified, each encoded by a separate gene. A recent review by Burton *et al.*, (1995 The Plant Journal 7, 3-15) has demonstrated that the two forms of SBE constitute distinct classes of the enzyme such that, in general, enzymes of the same class from different plants may exhibit greater similarity than enzymes of different classes from the same plant. In their review, Burton *et al.* termed the two respective enzyme families class "A" and class "B", and the reader is referred thereto (and to the references cited therein) for a detailed discussion of the distinctions between the two classes. One general distinction of note would appear to be the presence, in class A SBE molecules, of a flexible N-terminal domain, which is not found in class B molecules. The distinctions noted by Burton *et al.* are relied on herein to define class A and class B SBE

molecules, which terms are to be interpreted accordingly.

Many organisations have interests in obtaining modified Cassava starches by means of genetic modification. This is impossible to achieve however, unless the plant is amenable to transformation and regeneration, and the starch biosynthesis genes which are to be targeted for modification must be cloned. The production of transgenic cassava plants has only recently been demonstrated (Taylor *et al.*, 1996 Nature Biotechnology 14, 726-730; Schöpke *et al.*, 1996 Nature Biotechnology 14, 731-735; and Li *et al.*, 1996 Nature Biotechnology 14, 736-740). The present invention concerns the identification, cloning and sequencing of a starch biosynthetic gene from Cassava, suitable as a target for genetic manipulation.

Summary of the Invention

In a first aspect the invention provides a nucleic acid sequence encoding a polypeptide having starch branching enzyme (SBE) activity, the polypeptide comprising an effective portion of the amino acid sequences shown in Figure 4 or Figure 13. The nucleic acid is conveniently in substantial isolation, especially in isolation from other naturally associated nucleic acid sequences.

An "effective portion" of the amino acid sequences may be defined as a portion which retains sufficient SBE activity when expressed in *E. coli* KV832 to complement the branching enzyme mutation therein. The amino acid sequences shown in Figures 4 and 13 include the N terminal transit peptide, which comprises about the first 50 amino acid residues. As those skilled in the art will be well aware, such a transit peptide is not essential for SBE activity. Thus the mature polypeptide, lacking a transit peptide, may be considered as one example of an effective portion of the amino acid sequence shown in Figure 4 or Figure 13.

Other effective portions may be obtained by effecting minor deletions in the amino acid sequence, whilst substantially preserving SBE activity. Comparison with known class A SBE sequences, with the benefit of the disclosure herein, will enable those skilled in the

art to identify regions of the polypeptide which are less well conserved and so amenable to minor deletion, or amino acid substitution (particularly, conservative amino acid substitution) whilst substantially preserving SBE activity. Such less well-conserved regions are generally found in the N terminal amino acid residues (up to the triple proline "elbow" at residues 138-140 in Figure 4 and up to the proline elbow at residues 143-145 in Figure 13) and in the last 50 residues or so of the C terminal, and in particular in the acidic tail of the C terminal.

Conveniently the nucleic acid sequence is obtainable from cassava, preferably obtained therefrom, and typically encodes a polypeptide obtainable from cassava. In a particular embodiment, the encoded polypeptide may have the amino acid sequence NSKH at about position 697 (in relation to Figure 4), which sequence appears peculiar to an isoform of the SBE class A enzyme of cassava, other class A SBE enzymes having the conserved sequence DA D/E Y (Burton *et al.*, 1995 cited above).

In a particular aspect of the invention there is provided a nucleic acid comprising a portion of nucleotides 21 to 2531 of the nucleic acid sequence shown in Figure 4, or a functionally equivalent nucleic acid sequence. Such functionally equivalent nucleic acid sequences include, but are not limited to, those sequences which encode substantially the same amino acid sequence but which differ in nucleotide sequence from that shown in Figure 4 by virtue of the degeneracy of the genetic code. For example, a nucleic acid sequence may be altered (e.g. "codon optimised") for expression in a host other than cassava, such that the nucleotide sequence differs substantially whilst the amino acid sequence of the encoded polypeptide is unchanged. Other functionally equivalent nucleic acid sequences are those which will hybridise under stringent hybridisation conditions (e.g. as described by Sambrook *et al.*, Molecular Cloning. A Laboratory Manual. CSH, i.e. washing with 0.1xSSC, 0.5% SDS at 68°C) with the sequence shown in Figure 4. Figure 10 shows a functionally equivalent sequence designated "125 + 94", which includes a region corresponding to the 3' coding portion of the sequence in Figure 4. Figure 13 shows a functionally equivalent sequence which comprises a second complete SBE coding sequence (the SBE-derived sequence is from nucleotides 35 to 2760, of which the coding sequence is nucleotides 131-2677, the rest of the sequence in the figure is vector-derived).

Functionally equivalent DNA sequences will preferably comprise at least 200-300bp, more preferably 300-600bp, and will exhibit at least 88% identity (more preferably at least 90%, and most preferably at least 95% identity) with the corresponding region of the DNA sequence shown in figures 4 or 10. Those skilled in the art will readily be able to conduct a sequence alignment between the putative functionally equivalent sequence and those detailed in Figures 4 or 10 - the identity of the two sequences is to be compared in those regions which are aligned by standard computer software, which aligns corresponding regions of the sequences.

In particular embodiments the nucleic acid sequence may alternatively comprise a 5' and/or a 3' untranslated region ("UTR"), examples of which are shown in Figures 2 and 4. Figure 9 includes a 3' UTR, as nucleotides 688-1044 and Figure 10 includes 3' UTR as nucleotides 1507-1900 (which nucleotides correspond to the first base after the "stop" codon to the base immediately preceding the poly (A) tail). Any one of the sequences defined above, or a functional equivalent thereof (as defined by hybridisation properties, as set out in the preceding paragraph), could be useful in sense or anti-sense inhibition of corresponding genes, as will be apparent to those skilled in the art. It will also be apparent to those skilled in the art that such regions may be modified so as to optimise expression in a particular type of host cell and that the 5' and/or 3' UTRs could be used in isolation, or in combination with a coding portion of the sequence of the invention. Similarly, a coding portion could be used without a 5' or a 3' UTR if desired.

In a further aspect, the invention provides a replicable nucleic acid construct comprising any one of the nucleic acid sequences defined above. The construct will typically comprise a selectable marker and may allow for expression of the nucleic acid sequence of the invention. Conveniently the vector will comprise a promoter (especially a promoter sequence operable in a plant and/or a promoter operable in a bacterial cell) and one or more regulatory signals known to those skilled in the art.

In another aspect the invention provides a polypeptide having SBE activity, the polypeptide comprising an effective portion of the amino acid sequence shown in Figure 4 or Figure 13. The polypeptide is conveniently one obtainable from cassava, although it may be

derived using recombinant DNA techniques. The polypeptide is preferably in substantial isolation from other polypeptides of plant origin, and more preferably in substantial isolation from any other polypeptides. The polypeptide may have amino acid residues NSKH at about position 697 (in the sequence shown in Figure 4), instead of the sequence DA D/E Y found in other SBE class A polypeptides. The polypeptide may be used in a method of modifying starch *in vitro*, the method comprising treating starch under suitable conditions (of temperature, pH etc.) with an effective amount of the polypeptide.

Those skilled in the art will appreciate that the disclosure of the present specification can be utilised in a number of ways. In particular, the characteristics of a host cell may be altered by recombinant DNA techniques. Thus, in a further aspect, there is provided a method by which a host cell may be altered by introduction of a nucleic acid sequence comprising at least 200bp and exhibiting at least 88% sequence identity (more preferably at least 90%, and most preferably at least 95% identity) with the corresponding region of the DNA sequence shown in Figures 4, 9, 10 or 13, operably linked in the sense or (preferably) in the anti-sense orientation to a suitable promoter active in the host cell, and causing transcription of the introduced nucleic acid sequence, said transcript and/or the translation product thereof being sufficient to interfere with the expression of a homologous gene naturally present in said host cell, which homologous gene encodes a polypeptide having SBE activity. The altered host cell is typically a plant cell, such as a cell of a cassava, banana, potato, sweet potato, tomato, pea, wheat, barley, oat, maize, or rice plant.

Desirably the method further comprises the introduction of one or more nucleic acid sequences which are effective in interfering with the expression of other homologous gene or genes naturally present in the host cell. Such other genes whose expression is inhibited may be involved in starch biosynthesis (e.g. an SBE I gene), or may be unrelated to SBE II.

Those skilled in the art will be aware that both anti-sense inhibition, and "sense suppression" of expression of genes, especially plant genes, has been demonstrated (e.g. Matzke & Matzke 1995 Plant Physiol. 107, 679-685).

It is believed that antisense methods are mainly operable by the production of antisense mRNA which hybridises to the sense mRNA, preventing its translation into functional polypeptide, possibly by causing the hybrid RNA to be degraded (e.g. Sheehy *et al.*, 1988 PNAS *85*, 8805-8809; Van der Krol *et al.*, Mol. Gen. Genet. *220*, 204-212). Sense suppression also requires homology between the introduced sequence and the target gene, but the exact mechanism is unclear. It is apparent however that, in relation to both antisense and sense suppression, neither a full length nucleotide sequence, nor a "native" sequence is essential. Preferably the nucleic acid sequence used in the method will comprise at least 200-300bp, more preferably at least 300-600bp, of the full length sequence, but by simple trial and error other fragments (smaller or larger) may be found which are functional in altering the characteristics of the plant. It is also known that untranslated portions of sequence can suffice to inhibit expression of the homologous gene - coding portions may be present within the introduced sequence, but they do not appear to be essential under all circumstances.

The inventors have discovered that there are at least two class A SBE genes in cassava. A fragment of a second gene has been isolated, which fragment directs the expression of the C terminal 481 amino acids of cassava class A SBE (see Figure 10) and comprises a 3' untranslated region. Subsequently, a complete clone of the second gene was also recovered (see Figure 12). The coding portions of the two genes show some slight differences, and the second SBE gene may be considered as functionally equivalent to the corresponding portion of the nucleotide sequence shown in Figure 4. However, the 3' untranslated regions of the two genes show marked differences. Thus the method of altering a host cell may comprise the use of a sufficient portion of either gene so as to inhibit the expression of the naturally occurring homologous gene. Conveniently, a portion of nucleotide sequence is employed which is conserved between both genes. Alternatively, sufficient portions of both genes may be employed, typically using a single construct to direct the transcription of both introduced sequences.

In addition, as explained above, it may be desired to cause inhibition of expression of the class B SBE (i.e. SBE I) in the same host cell. A number of class B SBE gene sequences are known, including portions of the cassava class B SBE (Salehuzzaman *et al.*, 1994

Plant Science 98, 53-62) and any one of these may prove suitable. Preferably the sequence used is that which derives from the host cell sought to be altered (e.g. when altering the characteristics of a cassava plant cell, it is generally preferred to use sense or anti-sense sequences corresponding exactly to at least portions of the cassava gene whose expression is sought to be inhibited).

In a further aspect the invention provides an altered host cell, into which has been introduced a nucleic acid sequence comprising at least 200bp and exhibiting at least 88% sequence identity (more preferably at least 90%, and most preferably at least 95% identity) with the corresponding region of the DNA sequence shown in Figures 4, 9, 10 or 13, operably linked in the sense or anti-sense orientation to a suitable promoter, said host cell comprising a natural gene sharing sequence homology with the introduced sequence.

The host cell may be a micro-organism (such as a bacterial, fungal or yeast cell) or a plant cell. Conveniently the host cell altered by the method is a cell of a cassava plant, or another plant with starch storage reserves, such as banana, potato, sweet potato, tomato, pea, wheat, barley, oat, maize, or rice plant. Typically the sequence will be introduced in a nucleic acid construct, by way of transformation, transduction, micro-injection or other method known to those skilled in the art. The invention also provides for a plant into which has been introduced a nucleic acid sequence of the invention, or the progeny of such a plant.

The altered plant cell will preferably be grown into an altered plant, using techniques of plant growth and cultivation well-known to those skilled in the art of re-generating plantlets from plant cells.

The invention also provides a method of obtaining starch from an altered plant, the plant being obtained by the method defined above. Starch may be extracted from the plant by any of the known techniques (e.g. milling). The invention further provides starch obtainable from a plant altered by the method defined above, the starch having altered properties compared to starch extracted from an equivalent but unaltered plant. Conveniently the altered starch is obtained from an altered plant selected from the group

consisting of cassava, potato, pea, tomato, maize, wheat, barley, oat, sweet potato and rice. Typically the altered starch will have increased amylose content.

The invention will now be further described by way of illustrative examples and with reference to the accompanying drawings, in which:-

Figure 1 is a schematic illustration of the cloning strategy for cassava SBE II. The top line represents the size of a full length clone with distances in kilobases (kb) and arrows representing oligonucleotides (rightward pointing arrows are sense strand, leftward are on opposite strand). The long thick arrow is the open reading frame with start and stop codons shown. Below this are shown the 3' RACE, 5' RACE and PCR clones identified either by the plasmid name (shown in brackets above the line) or the clone number (shown to the left of the clone) for the 5' RACE only. Also shown (by an x) in the 5' RACE clones are positions of small deletions or introns.

Figure 2 shows the DNA sequence and predicted ORF of csbe2con.seq. This sequence is a consensus of 3' RACE pSJ94 and 5' RACE clones 27/9,11 and 28. The first 64 base pairs are derived from the RoRIdT17 adaptor primer/dT tail followed by the SBE sequence. The one long open reading frame is shown in one letter code below the double strand DNA sequence. Also shown is the upstream ORF (MQL...LPW).

Figure 3 shows an alignment of the 5' region of cassava SBE II csbe2con and pSJ99 (clones 20 and 35) DNA sequences. Differences from the consensus sequence are shaded.

Figure 4 shows the DNA sequence and predicted ORF of full length cassava SBE II tuber cDNA in pSJ107. The sequence shown is from the CSBE214 to the CSBE218 oligonucleotide. The DNA sequence is sequence ID No. 28 in the attached sequence listing; the amino acid sequence is Seq ID No. 29.

Figure 5 shows an alignment of 3' region of cassava SBE II pSJ116 and 125+94 DNA sequences. The top line is the 125 + 94 sequence and the bottom SJ116 sequence. Identical nucleotides are indicated by the same letter in the middle line. differences are

indicated by a gap, and dashed lines indicate gaps introduced to optimise alignment.

Figure 6 shows an alignment of carboxy terminal region of pSJ116 and 125+94 protein sequences. The top sequence is from 125+94 and the bottom from pSJ116. Identical amino acid residues are shown with the same letter, conserved changes with a colon and neutral changes with a period.

Figure 7 shows a phylogenetic tree of starch branching enzyme proteins. The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences (units indicate the number of substitution events). Dotted lines indicate a negative branch length because of averaging the tree. Zmcon12.pro is maize SBE II. psstb1.pro is pea SBE I (Bhattacharyya *et al* 1990 Cell **60**, 115-121) and atsbe2-1 & 2-2.pro are two SBE II proteins from *Arabidopsis thalania* (Fisher *et al* 1996 Plant Mol. Biol. 30, 97-108). SJ107.pro is representative of a cassava SBE II sequence, and potsbe2.pro is a potato SBE II sequence known to the inventors.

Figure 8 is an alignment of SBE II proteins. Protein sequences are indicated in one letter code. The top line represents the consensus sequence, below which is shown the consensus ruler and the individual SBE II sequences. Residues matching the consensus are shaded. Dashes represent gaps introduced to optimise alignment. Sequence identities are shown at the right of the figure and are as Figure 7, except that SJ107.pro is cassava SBE II.

Figure 9 shows the DNA sequence and predicted ORF of a cassava SBE II cDNA isolated by 3' RACE (plasmid pSJ 101).

Figure 10 shows the consensus DNA sequence and predicted ORF of a second cassava SBE II cDNA isolated by 3' and 5' RACE (sequence designated 125+94 is from plasmid pSJ125 and pSJ94, spliced at the CSBE217 oligo sequence).

Figure 11 is a schematic diagram of the plant transformation vector pSJ64. The black line represents the DNA sequence. The hashed line represents the bacterial plasmid backbone

(containing the origin of replication and bacterial selection marker) and is not shown in full. The filled triangles represent the T-DNA borders (RB = right border, LB = left border). Relevant restriction enzyme sites are shown above the black line with the approximate distances (in kiloobases) betwen sites marked by an asterisk shown underneath. The thinnest arrows represent polyadenylation signals (pAnos = nopaline synthase, pAg7 = Agrobacterium gene 7), the intermediate arrows represent protein coding regions (SBE II = cassava SBE II, HYG = hygromycin resistance gene) and the thick arrows represent promoter regions (P-2x35S = double CaMV 35S promoter, P-nos = nopaline synthase promoter).

Figure 12 is a schematic illustration of the cloning strategy used to isolate a second cassava SBE II gene. The top line represents the size of a full length clone with distances in kilobases (kb) and arrows representing oligonucleotides (rightward pointing arrows are sense strand, leftward are on opposite strand). The long thick arrow is the open reading frame with start and stop codons shown. Below this are shown the 3' RACE, 5' RACE and PCR clones identified either by the plasmid name (shown in brackets above the line) or the clone number (shown to the right of the clone).

Figure 13 shows the DNA sequence and predicted ORF of a second full length cassava SBE II tuber cDNA in pSJ146. Nucleotides 35-2760 are SBE II sequence and the remainder are from the pT7Blue vector. The DNA sequence of Figure 13 is Seq ID No. 30, and the amino acid sequence is Seq ID No. 31, in the attached sequence listing.

Example 1

This example relates to the isolation and cloning of SBE II sequences from cassava.

Recombinant DNA manipulations

Standard procedures were performed essentially according to Sambrook *et al.* (1989 Molecular cloning A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). DNA sequencing was performed on an ABI automated DNA sequencer and sequences manipulated using DNASTAR software for the Macintosh.

Rapid Amplification of cDNA ends (RACE) and PCR conditions

5' and 3' RACE were performed essentially according to Frohman *et al.*, (1988 Proc. Natl. Acad. Sci. USA 85, 8998-9002) but with the following modifications.

For 3' RACE, 5 μg of total RNA was reverse transcribed using 5 pmol of the RACE adaptor RoRidT17 as primer and Stratascript RNAse H- reverse transcriptase (50 U) in a 50 μl reaction according to the manufacturer's instructions (Stratagene). The reaction was incubated for 1 hour at 37°C and then diluted to 200 μl with TE (10 mM Tris HCl, 1 mM EDTA) pH 8 and stored at 4°C. 2.5 μl of this cDNA was used in a 25 μl PCR reaction with 12.5 pmol of SBE A and Ro primers for 30 cycles of 94°C 45 sec, 50°C 25 sec, 72°C 1 min 30 sec. A second round of PCR (25 cycles) was performed using 1 μl of this reaction as template in a 50 μl reaction under the same conditions. Amplified products were separated by agarose gel electrophoresis and cloned into the pT7Blue vector (Invitrogen).

For the first round of 5' RACE, 5 μg of total leaf RNA was reverse transcribed as described above using 10 pmol of the SBE II gene specific primer CSBE22. This primer was removed from the reaction by diluting to 500 μl with TE and centrifuging twice through a centricon 100 microconcentrator. The concentrated cDNA was then dA-tailed with 9U of terminal deoxynucleotide transferase and 50 μM dATP in a 20 μl reaction in buffer supplied by the manufacturer (BRL). The reaction was incubated for 10 min at 37°C and 5 min at 65°C and then diluted to 200 μl with TE pH 8. PCR was performed in a 50 μl volume using 5μl of tailed cDNA, 2.5 pmol of RoRidT17 and 25 pmol of Ro and CSBE24 primers for 30 cycles of 94°C 45 sec, 55°C 25 sec, 72°C 3 min. Amplified products were separated on a 1% TAE agarose gel, cut out, 200μl of TE was added and melted at 99°C for 10 min. Five μl of this was re-amplified in a 50 μl volume using CSBE25 and Ri as primers and 25 cycles of 94°C 45 sec, 55°C 25 sec, 72°C 1 min 30 sec. Amplified fragments were separated on a 1% TAE agarose gel, purified on DEAE paper and cloned into pT7Blue.

The second round of 5' RACE was performed using CSBE28 and 29 primers in the first and second round PCR reactions respectively using a new A-tailed cDNA library primed

with CSBE27.

A third round of 5' RACE was performed on the same CSBE27 primed cDNA.

Repeat 3' RACE and PCR Cloning

The 3' RACE library (RoRidT17 primed leaf RNA) was used as a template. The first PCR reaction was diluted 1:20 and 1 μl was used in a 50 μl PCR reaction with SBE A and Ri primers and the products were cloned into pT7Blue. The cloned PCR products were screened for the presence or absence of the CSBE23 oligo by colony PCR.

A full length cDNA of cassava SBE II was isolated by PCR from leaf or root cDNA (RoRidT17 primed) using primers CSBE214 and CSBE218 from 2.5 μl of cDNA in a 25 μl reaction and 30 cycles of 94°C 45 sec, 55°C 25 sec, 72°C 2 min.

Complementation of *E. coli* mutant KV832

SBE II containing plasmids were transformed into the branching enzyme deficient mutant *E. coli* KV832 (Keil *et al.*, 1987 Mol. Gen. Genet. **207**, 294-301) and cells grown on solid PYG media (0.85 % KH_2PO_4, 1.1 % K_2HPO_4, 0.6 % yeast extract) containing 1.0 % glucose. To test for complementation, a loop of cells was scraped off and resuspended in 150 μL water to which was added 15 μL of Lugol's solution (2 g KI and 1 g I_2 per 300 ml water).

RNA isolation

RNA was isolated from cassava plants by the method of Logemann (1987 Anal. Biochem. 163, 21-26). Leaf RNA was isolated from 0.5 gm of in vitro grown plant tissue. The total yield was 300 μg. Three month old roots (88 gm) were used for isolation of root RNA).

SBE II specific oligonucleotides

SBE A	ATGGACAAGGATATGTATGA	(Seq ID No. 1)
CSBE21	GGTTTCATGACTTCTGAGCA	(Seq ID No. 2)

CSBE22	TGCTCAGAAGTCATGAAACC	(Seq ID No. 3)
CSBE23	TCCAGTCTCAATATACGTCG	(Seq ID No. 4)
CSBE24	AGGAGTAGATGGTCTGTCGA	(Seq ID No. 5)
CSBE25	TCATACATATCCTTGTCCAT	(Seq ID No. 6)
CSBE26	GGGTGACTTCAATGATGTAC	(Seq ID No. 7)
CSBE27	GGTGTACATCATTGAAGTCA	(Seq ID No. 8)
CSBE28	AATTACTGGCTCCGTACTAC	(Seq ID No. 9)
CSBE29	CATTCCAACGTGCGACTCAT	(Seq ID No. 10)
CSBE210	TACCGGTAATCTAGGTGTTG	(Seq ID No. 11)
CSBE211	GGACCTTGGTTTAGATCCAA	(Seq ID No. 12)
CSBE212	ATGAGTCGCACGTTGGAATG	(Seq ID No. 13)
CSBE213	CAACACCTAGATTACCGGTA	(Seq ID No. 14)
CSBE214	TTAGTTGCGTCAGTTCTCAC	(Seq ID No. 15)
CSBE215	AATATCTATCTCAGCCGGAG	(Seq ID No. 16)
CSBE216	ATCTTAGATAGTCTGCATCA	(Seq ID No. 17)
CSBE217	TGGTTGTTCCCTGGAATTAC	(Seq ID No. 18)
CSBE218	TGCAAGGACCGTGACATCAA	(Seq ID No. 19)

RESULTS

Cloning of a SBE II gene from cassava leaf

The strategy for cloning a full length cDNA of starch branching enzyme II of cassava is shown in Figure 1. A comparison of several SBE II (class A) SBE DNA sequences identified a 23 bp region which appears to be completely conserved among most genes (data not shown) and is positioned about one kilobase upstream from the 3' end of the gene. An oligonucleotide primer (designated SBE A) was made to this sequence and used to isolate a partial cDNA clone by 3' RACE PCR from first strand leaf cDNA as illustrated in Figure 1. An approximately 1100 bp band was amplified, cloned into pT7Blue vector and sequenced. This clone was designated pSJ94 and contained a 1120 bp insert starting with the SBE A oligo and ending with a polyA tail. There was a predicted open reading frame of 235 amino acids which was highly homologous (79% identical) to a potato SBE II also isolated by the inventors (data not shown) suggesting that this clone represented a class A (SBE II) gene.

To obtain the sequence of a full length clone nested primers were made complementary to the 5' end of this sequence and used in 5' RACE PCR to isolate clones from the 5' region of the gene. A total of three rounds of 5' RACE was needed to determine the sequence of the complete gene (i.e. one that has a predicted long ORF preceded by stop codons). It should be noted that during this cloning process several clones (# 23, 9, 16) were obtained that had small deletions and in one case (clone 23) there was also a small (120 bp) intron present. These occurrences are not uncommon and probably arise through errors in the PCR process and/or reverse transcription of incompletely processed RNA (heterogeneous nuclear RNA).

The overlapping cDNA fragments could be assembled into a contiguous 3 kb sequence (designated csbe2con.seq) which contained one long predicted ORF as shown in Figure 2. Several clones in the last round of 5' RACE were obtained which included sequence of the untranslated leader (UTL). All of these clones had an ORF (42 amino acids) 46 bp upstream and out of frame with that of the long ORF.

There is more than one SBE II gene in cassava

In order to determine if the assembled sequence represented that of a single gene, attempts were made to recover by PCR a full length SBE II gene using primers CSBE214 and CSBE23 at the 5' and 3' ends of the csbe2con sequence respectively. All attempts were unsuccessful using either leaf or root cDNA as template. The PCR was therefore repeated with either the 5'- or 3'- most primer and complementary primers along the length of the SBE II gene to determine the size of the largest fragment that could be amplified. With the CSBE214 primer, fragments could be amplified using primers 210, 28, 27 and 22 in order of increasing distance, the latter primer pair amplifying a 2.2 kb band. With the 3' primer CSBE23, only primer pairs with 21 and 26 gave amplification products, the latter being about 1200 bp. These results suggest that the original 3' RACE clone (pSJ94) is derived from a different SBE II gene than the rest of the 5' RACE clones even though the two largest PCR fragments (214+22 and 26+23) overlap by 750 bp and share several primer sites. It is likely that the sequence of the two genes starts to diverge around the CSBE22 primer site such that the 3' end of the corresponding gene does not contain the 23 primer and is not therefore able to amplify a cDNA when used with the 214 primer.

To confirm this, the sequence of the longest 5' PCR fragment (214+22) from two clones (#20 designated pSJ99. & #35) was determined and compared to the consensus sequence csbe2con as shown in Figure 3. The first 2000 bases are nearly identical (the single base changes might well be PCR errors), however the consensus sequence is significantly different after this. This region corresponds to the original 3' RACE fragment pSJ94 (SBE A + Ri adaptor) and provided evidence that there may be more than one SBE II gene in cassava.

The 3' end corresponding to pSJ99 was therefore cloned as follows: 3' RACE PCR was performed on leaf cDNA using the SBE A oligo as the gene specific primer so that all SBE II genes would be amplified. The cloned DNA fragments were then screened for the presence or absence of the CSBE23 primer by PCR. Two out of 15 clones were positive with the SBE A + Ri primer pair but negative with SBE A + CSBE23 primers. The sequence of these two clones (designated pSJ101, as shown in Figure 9) demonstrated that they were indeed from an SBE II gene and that they were different from pSJ94. However the overlapping region of pSJ101 (the 3' clone) and pSJ99 (the 5' clone) was identical suggesting that they were derived from the same gene.

To confirm this a primer (CSBE218) was made to a region in the 3' UTR (untranslated region) of pSJ101 and used in combination with CSBE214 primer to recover by PCR a full length cDNA from both leaf and root cDNA. These clones were sequenced and designated pSJ106 & pSJ107 respectively. The sequence and predicted ORF of pSJ107 is shown in Figure 4. The long ORF in plasmid pSJ106 was found to be interrupted by a stop codon (presumably introduced in the PCR process) approximately 1 kb from the 3' end of the gene, therefore another cDNA clone (designated pSJ116) was amplified in a separate reaction, cloned and sequenced. This clone had an intact ORF (data not shown). There were only a few differences in these two sequences (in the transit peptide aa 27- 41: YRRTSSCLSFNFKEA to DRRTSSCLSFIFKKAA and L831 in pSJ107 to V in pSJ116 respectively).

An additional 740bp of sequence of the gene corresponding to the pSJ94 clone was isolated by 5' RACE using the primers CSBE216 and 217, and was designated pSJ125.

This sequence was combined with that of pSJ94 to form a consensus sequence "125 + 94", as shown in Figure 10. The sequence of this second gene is about 90% identical at the DNA and protein level to pSJ116, as shown in Figure 5 and 6, and is clearly a second form of SBE II in cassava. The 3' untranslated regions of the two genes are not related (data not shown).

It was also determined that the full length cassava SBE II genes (from both leaf and tuber) actually encode for active starch branching enzymes since the cloned genes were able to complement the glycogen branching enzyme deficient *E. coli* mutant KV832.

Main Findings

1) A full length cDNA clone of a starch branching enzyme II (SBE II) gene has been cloned from leaves and starch storing roots of cassava. This cDNA encodes a 836 amino acid protein (Mr 95 Kd) and is 86 % identical to pea SBE I over the central conserved domain, although the level of sequence identity over the entire coding region is lower than 86%.

2) There is more than one SBE II gene in cassava as a second partial SBE II cDNA was isolated which differs slightly in the protein coding region from the first gene and has no homology in the 3' untranslated region.

3) The isolated full length cDNA from both leaves and roots encodes an active SBE as it complements an *E. coli* mutant deficient in glycogen branching enzyme as assayed by iodine staining.

We have shown that there are SBE II (Class A) gene sequences present in the cassava genome by isolating cDNA fragments using 3' and 5' RACE. From these cDNA fragments a consensus sequence of over 3 kb could be compiled which contained one long open reading frame (Figure 2) which is highly homologous to other SBE II (class A) genes (data not shown). It is likely that the consensus sequence does not represent that of a single gene since attempts to PCR a full length gene using primers at the 5' and 3' ends of this sequence were not successful. In fact screening of a number of leaf derived 3'

RACE cDNAs showed that a second SBE II gene (clone designated pSJ101) was also expressed which is highly homologous within the coding region to the originally isolated cDNA (pSJ94) but has a different 3' UTR. A full length SBE II gene was isolated from leaves and roots by PCR using a new primer to the 3' end of this sequence and the original sequence at the 5' end of the consensus sequence. If the frequency of clones isolated by 3' RACE PCR reflects the abundance of the mRNA levels then this full length gene may be expressed at lower levels in the leaf than the pSJ94 clone (2 out of 15 were the former class, 13/15 the latter). It should be noted that each class is expressed in both leaves and roots as judged by PCR (data not shown). Sequence analysis of the predicted ORF of the leaf and root genes showed only a few differences (4 amino acid changes and one deletion) which could have arisen through PCR errors or, alternatively, there may be more than one nearly identical gene expressed in these tissues.

A comparison of all known SBE II protein sequences shows that the cassava SBE II gene is most closely related to the pea gene (Figure 8). The two proteins are 86.3% identical over a 686 amino acid range which extends from the triple proline "elbow" (Burton *et al.*, 1995 Plant J. 7, 3-15) to the conserved VVYA sequence immediately preceding the C-terminal extensions (data not shown). All SBE II proteins are conserved over this range in that they are at least 80% similar to each other. Remarkably however, the sequence conservation between the pea, potato and cassava SBE II proteins also extends to the N-terminal transit peptide, especially the first 12 amino acids of the precursor protein and the region surrounding the mature terminus of the pea protein (AKFSRDS). Because the proteins are so similar around this region it can be predicted that the mature terminus of the cassava SBE II protein is likely to be GKSSHES. The precursor has a predicted molecular mass of 96 kD and the mature protein a predicted molecule mass of 91.3 kD. The cassava SBE II has a short acidic tail at the C-terminal although this is not as long or as acidic as that found in the pea or potato proteins. The significance of this acidic tail, if any, remains to be determined. One notable difference between the amino acid sequence of cassava SBE II and all other SBE II proteins is the presence of the sequence NSKH at around position 697 instead of the conserved sequence DAD/EY. Although this conserved region forms part of a predicted α-helix (number 8) of the catalytic $(\beta/\alpha)_8$ barrel domain (Burton et al 1995 cited previously), this difference does not abolish the SBE

activity of the cassava protein as this gene can still complement the glycogen branching deletion mutant of *E. coli*. It may however affect the specificity of the protein. An interesting point is that the other cassava SBE II clone pSJ94 has the conserved sequence DADY.

One other point of interest concerning the sequence of the SBE II gene is the presence of an upstream ATG in the 5' UTR. This ATG could initiate a small peptide of 42 amino acids which would terminate downstream of the predicted initiating methionine codon of the SBE II precursor. If this does occur then the translation of the SBE II protein from this mRNA is likely to be inefficient as ribosomes normally initiate at the 5' most ATG in the mRNA. However the first ATG is in a poorer Kozak context than the SBE II initiator and it may be too close to the 5' end of the message to initiate efficiently (14 nucleotides) thus allowing initiation to occur at the correct ATG.

In conclusion we have shown that cassava does have SBE II gene sequences, that they are expressed in both leaves and tubers and that more than one gene exists.

Example 2

Cloning of a second full length cassava SBE II gene

Methods

Oligonucleotides

CSBE219	CTTTATCTATTAAAGACTTC	(Seq ID No. 20)
CSBE220	CAAAAAAGTTTGTGACATGG	(Seq ID No. 21)
CSBE221	TCACTTTTTCCAATGCTAAT	(Seq ID No. 22)
CSBE222	TCTCATGCAATGGAACCGAC	(Seq ID No. 23)
CSBE223	CAGATGTCCTGACTCGGAAT	(Seq ID No. 24)
CSBE224	ATTCCGAGTCAGGACATCTG	(Seq ID No. 25)
CSBE225	CGCATTTCTCGCTATTGCTT	(Seq ID No. 26)
CSBE226	CACAGGCCCAAGTGAAGAAT	(Seq ID No. 27)

The 5' end of the gene corresponding to the 3'RACE clone pSJ94 was isolated in three

rounds of 5'RACE. Prior to performing the first round of 5' RACE, 5 μg of total leaf RNA was reverse transcribed in a 20 μl reaction using conditions as decribed by the manufacturer (Superscript enzyme, BRL) and 10 pmol of the SBE II gene specific primer CSBE23. Primers were then removed and the cDNA tailed with dATP as described above. The first round of 5'RACE used primers CSBE216 and Ro. This PCR reaction was diluted 1:20 and used as a template for a second round of amplification using primers CSBE217 and Ri. The gene specific primers were designed so that they would preferentially hybridise to the SBE II sequence in pSJ94. Amplified products appeared as a smear of approximately 600-1200 bp when subjected to electrophoresis on a 1% TAE agarose gel.

This smear was excised and DNA purified using a Qiaquick column (Qiagen) before ligation to the pT7Blue vector. Several clones were sequenced and clone #7 was designated pSJ125. New primers (CSBE219 and 220) were designed to hybridise to the 5' end of pSJ125 and a second round of 5'RACE was performed using the same CSBE23 primed library. Two fragments of 600 and 800 bp were cloned and sequenced (clones 13,17). Primers CSBE221 and 222 were designed to hybridise to the 5' sequence of the longest clone (#13) and a third round of 5' RACE was performed on a new library (5 μg total leaf RNA reverse transcribed with Superscript using CSBE220 as primer and then dATP tailed with TdT from Boehringer Mannheim). Fragments of approximately 500 bp were amplified, cloned and sequenced. Clone #13, was designated pSJ143. The process is illustrated schematically in Figure 12.

To isolate a full length gene as a contiguous sequence, a new primer (CSBE225) was designed to hybridise to the 5' end of clone pSJ143 and used with one of the primers (CSBE226 or 23) in the 3' end of clone pSJ94, in a PCR reaction using RoRidT17 primed leaf cDNA as template. Use of primer CSBE226 resulted in production of Clone #2 (designated pSJ144), and use of primer CSBE23 resulted in production of Clones #10 and 13 (designated pSJ145 and pSJ146 respectively). Only pSJ146 was sequenced fully.

Results

Isolation of a second full length cassava SBE II gene

A full length clone for a second SBE II gene was isolated by extending the sequence of pSJ94 in three rounds of 5' RACE as illustrated schematically in Figure 12. In each round of 5' RACE, primers were designed that would preferentially hybridise to the new sequence rather than to the gene represented by pSJ116. In the final round of 5' RACE, three clones were obtained that had the initiating methione codon, and none of these had upstream ATGs. The overlapping cDNA fragments (sequences of the 5'RACE clones pSJ143, 13, pSJ125 and the 3'RACE clone pSJ94) could be assembled into a consensus sequence of approximately 3 kb which was designated csbe2-2.seq. This sequence contained one long ORF with a predicted size of 848 aa (M_r 97 kDa). The full length gene was then isolated as a contiguous sequence by PCR amplification from RoRidT17 primed leaf cDNA using primers at the 5' (CSBE225) and 3' (CSBE23 or CSBE226) ends of the RACE clones. One clone, designated pSJ146, was sequenced and the restriction map is shown along with the predicted amino acid sequence in Figure 13.

<u>Sequence homologies between SBE II genes</u>

The two cassava genes (pSJ116 and pSJ146) share 88.8% identity at the DNA level over the entire coding region (data not shown). The homology extends about 50 bases outside of this region but beyond this the untranslated regions show no similarity (data not shown). At the protein level the two genes show 86% identity over the entire ORF (data not shown). The two genes are more closely related to each other than to any other SBE II. Between species, the pea SBE I shows the most homology to the cassava SBE II genes.

Example 3

<u>Construction of plant transformation vectors and transformation of cassava with antisense starch branching enzyme genes.</u>

This example describes in detail how a portion of the SBE II gene isolated from cassava may be introduced into cassava plants to create transgenic plants with altered properties.

An 1100 bp *Hind* III - *Sac* I fragment of cassava SBE II (from plasmid pSJ94) was cloned into the *Hind* III - *Sac* I sites of the plant transformation vector pSJ64 (Figure 11). This placed the SBE II gene in an antisense orientation between the 2X 35S CaMV promoter

and the nopaline synthase polyadenylation signal. PSJ64 is a derivative o the binary vector pGPTV-HYG (Becker et al., 1992 Plant Molecular Biology 20: 1195-1197) modified by inclusion of an approximately 750 bp fragment of pJIT60 (Guerineau et al 1992 Plant Mol. Biol. 18, 815-818) containing the duplicated cauliflower mosaic virus (CaMV) 35S promoter (Cabb-JI strain, equivalent to nucleotides 7040 to 7376 duplicated upstream of 7040 to 7433, as described by Frank et al., 1980 Cell 21, 285-294) to replace the GUS coding sequence. A similar construct was made with the cassava SBE II sequence from plasmid pSJ101.

These plasmids are then introduced into Agrobacterium tumefaciens LBA4404 by a direct DNA uptake method (An et al. Binary vectors, In: Plant Molecular Biology Manual (ed embryos by selecting on hygromycin as described by Li et al. (1996, Nature Biotechnology 14, 736-740).

The term "comprise" and grammatical variations thereof such as "comprising" when used in the description or claims does not preclude the presence of additional features, integers, steps or components.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: National Starch and Chemical Investment Holding Corporation
(B) STREET: Suite 27. 501 Silverside Road
(C) CITY: Wilmington
(D) STATE: Delaware
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 19809

(ii) TITLE OF INVENTION: Improvements in or Relating to Starch Content of Plants

(iii) NUMBER OF SEQUENCES: 31

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH. 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGGACAAGG ATATGTATGA 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGTTTCATGA CTTCTGAGCA 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TGCTCAGAAG TCATGAAACC 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCCAGTCTCA ATATACGTCG 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGGAGTAGAT GGTCTGTCGA 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCATACATAT CCTTGTCCAT 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGGTGACTTC AATGATGTAC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGTGTACATC ATTGAAGTCA 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AATTACTGGC TCCGTACTAC 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CATTCCAACG TGCGACTCAT 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TACCGGTAAT CTAGGTGTTG 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGACCTTGGT TTAGATCCAA 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATGAGTCGCA CGTTGGAATG 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CAACACCTAG ATTACCGGTA 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TTAGTTGCGT CAGTTCTCAC 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AATATCTATC TCAGCCGGAG 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATCTTAGATA GTCTGCATCA 20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGGTTGTTCC CTGGAATTAC 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGCAAGGACC GTGACATCAA 20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CTTTATCTAT TAAAGACTTC 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAAAAAAGTT TGTGACATGG 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TCACTTTTTC CAATGCTAAT 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TCTCATGCAA TGGAACCGAC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CAGATGTCCT GACTCGGAAT 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ATTCCGAGTC AGGACATCTG 20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CGCATTTCTC GCTATTGCTT 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CACAGGCCCA AGTGAAGAAT 20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2588 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:21..2531

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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CTCTCTAACT TCTCAGCGAA ATG GGA CAC TAC ACC ATA TCA GGA ATA CGT     50
                      Met Gly His Tyr Thr Ile Ser Gly Ile Arg
                       1               5                  10
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TTT CCT TGT GCT CCA CTC TGC AAA TCT CAA TCT ACC GGC TTC CAT GGC      98
Phe Pro Cys Ala Pro Leu Cys Lys Ser Gln Ser Thr Gly Phe His Gly
                15                  20                  25

TAT CGG AGG ACC TCC TCT TGC CTT TCC TTC AAC TTC AAG GAG GCG TTT     146
Tyr Arg Arg Thr Ser Ser Cys Leu Ser Phe Asn Phe Lys Glu Ala Phe
            30                  35                  40

TCT AGG AGG GTC TTC TCT GGA AAG TCA TCT CAT GAA TCT GAC TCC TCA     194
Ser Arg Arg Val Phe Ser Gly Lys Ser Ser His Glu Ser Asp Ser Ser
        45                  50                  55

AAT GTA ATG GTC ACT GCT TCT AAA AGA GTC CTT CCT GAT GGT CGG ATT     242
Asn Val Met Val Thr Ala Ser Lys Arg Val Leu Pro Asp Gly Arg Ile
    60                  65                  70

GAA TGC TAT TCT TCT TCA ACA GAT CAA TTG GAA GCC CCT GGC ACA GTT     290
Glu Cys Tyr Ser Ser Ser Thr Asp Gln Leu Glu Ala Pro Gly Thr Val
75                  80                  85                  90

TCA GAA GAA TCC CAG GTG CTT ACT GAT GTT GAG AGT CTC ATT ATG GAT     338
Ser Glu Glu Ser Gln Val Leu Thr Asp Val Glu Ser Leu Ile Met Asp
                95                  100                 105

GAT AAG ATT GTT GAA GAT GAA GTA AAT AAA GAA TCT GTT CCA ATG CGG     386
Asp Lys Ile Val Glu Asp Glu Val Asn Lys Glu Ser Val Pro Met Arg
            110                 115                 120

GAG ACA GTT AGC ATC AGA AAA ATT GGA TCT AAA CCA AGG TCC ATT CCT     434
Glu Thr Val Ser Ile Arg Lys Ile Gly Ser Lys Pro Arg Ser Ile Pro
        125                 130                 135

CCA CCC GGC AGA GGG CAA AGA ATA TAT GAC ATA GAT CCA AGC TTG ACA     482
Pro Pro Gly Arg Gly Gln Arg Ile Tyr Asp Ile Asp Pro Ser Leu Thr
    140                 145                 150

GGC TTT CGT CAA CAC CTA GAT TAC CGG TAT TCA CAG TAC AAA AGA CTC     530
Gly Phe Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Arg Leu
155                 160                 165                 170

CGA GAA GAA ATT GAC AAG TAT GAA GGT AGT CTG GAT GCA TTT TCT CGT     578
Arg Glu Glu Ile Asp Lys Tyr Glu Gly Ser Leu Asp Ala Phe Ser Arg
                175                 180                 185

GGC TAT GAA AAG TTT GGT TTC TCA CGC AGT GAA ACA GGA ATA ACT TAT     626
Gly Tyr Glu Lys Phe Gly Phe Ser Arg Ser Glu Thr Gly Ile Thr Tyr
            190                 195                 200

AGA GAG TGG GCA CCA GGA GCT ACG TGG GCT GCA TTG ATT GGA GAT TTC     674
Arg Glu Trp Ala Pro Gly Ala Thr Trp Ala Ala Leu Ile Gly Asp Phe
        205                 210                 215

AAT AAC TGG AAT CCT AAT GCA GAT GTC ATG ACT CAG AAT GAG TGT GGT     722
Asn Asn Trp Asn Pro Asn Ala Asp Val Met Thr Gln Asn Glu Cys Gly
    220                 225                 230
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GTC TGG GAG ATC TTT TTG CCG AAT AAT GCA GAT GGT TCA CCA CCA ATT 770
Val Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Ser Pro Pro Ile
235 240 245 250

CCC CAT GGT TCT CGA GTA AAG ATA CGC ATG GAT ACT CCA TCT GGC AAC 818
Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Asn
255 260 265

AAA GAT TCT ATT CCT GCT TGG ATC AAG TTC TCA GTT CAA GCA CCA GGT 866
Lys Asp Ser Ile Pro Ala Trp Ile Lys Phe Ser Val Gln Ala Pro Gly
270 275 280

GAA CTC CCA TAT AAT GGC ATA TAC TAT GAT CCT CCC GAG GAG GAG AAG 914
Glu Leu Pro Tyr Asn Gly Ile Tyr Tyr Asp Pro Pro Glu Glu Glu Lys
285 290 295

TAT GTG TTC AAA AAT CCT CAG CCA AAG AGA CCA AAA TCA CTT CGG ATT 962
Tyr Val Phe Lys Asn Pro Gln Pro Lys Arg Pro Lys Ser Leu Arg Ile
300 305 310

TAT GAG TCG CAC GTT GGA ATG AGT AGT ACG GAG CCA GTA ATT AAC ACA 1010
Tyr Glu Ser His Val Gly Met Ser Ser Thr Glu Pro Val Ile Asn Thr
315 320 325 330

TAT GCC AAC TTT AGA GAT GAT GTG CTT CCT CGC ATC AAA AAG CTT GGC 1058
Tyr Ala Asn Phe Arg Asp Asp Val Leu Pro Arg Ile Lys Lys Leu Gly
335 340 345

TAC AAT GCT GTT CAG CTC ATG GCT ATT CAA GAG CAT TCA TAT TAT GCT 1106
Tyr Asn Ala Val Gln Leu Met Ala Ile Gln Glu His Ser Tyr Tyr Ala
350 355 360

AGT TTT GGG TAT CAC GTC ACA AAC TTT TAT GCA GCT AGC AGC CGA TTT 1154
Ser Phe Gly Tyr His Val Thr Asn Phe Tyr Ala Ala Ser Ser Arg Phe
365 370 375

GGA ACT CCT GAT GAT TTA AAG TCT CTA ATA GAT AAA GCT CAC GAG TTA 1202
Gly Thr Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala His Glu Leu
380 385 390

GGT CTT CTT GTT CTC ATG GAT ATT GTT CAT AGC CAT GCA TCA ACT AAT 1250
Gly Leu Leu Val Leu Met Asp Ile Val His Ser His Ala Ser Thr Asn
395 400 405 410

ACG TTG GAT GGG CTG AAT ATG TTT GAT GGT ACG GAT GGT CAC TAC TTT 1298
Thr Leu Asp Gly Leu Asn Met Phe Asp Gly Thr Asp Gly His Tyr Phe
415 420 425

CAC TCT GGA CCA CGG GGT CAT CAT TGG ATG TGG GAC TCT CGC CTT TTC 1346
His Ser Gly Pro Arg Gly His His Trp Met Trp Asp Ser Arg Leu Phe
430 435 440

AAC TAT GGG AGC TGG GAG GTT CTA AGG TTT CTT CTT TCA AAT GCA AGG 1394
Asn Tyr Gly Ser Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Ala Arg
445 450 455

TGG TGG TTG GAT GAG TAC AAG TTT GAT GGG TTC AGA TTT GAT GGG GTG 1442
Trp Trp Leu Asp Glu Tyr Lys Phe Asp Gly Phe Arg Phe Asp Gly Val
460 465 470

ACT TCA ATG ATG TAC ACC CAT CAT GGA TTG CAG GTA GAT TTT ACC GGC 1490
Thr Ser Met Met Tyr Thr His His Gly Leu Gln Val Asp Phe Thr Gly
475 480 485 490

AAC TAC AAT GAA TAC TTT GGA TAT GCA ACT GAT GTA GAT GCT GTG GTT 1538
Asn Tyr Asn Glu Tyr Phe Gly Tyr Ala Thr Asp Val Asp Ala Val Val
495 500 505

TAT TTG ATG CTG TTG AAT GAT ATG ATT CAT GGT CTC TTC CCA GAG GCT 1586
Tyr Leu Met Leu Leu Asn Asp Met Ile His Gly Leu Phe Pro Glu Ala
510 515 520

GTC ACC ATT GGT GAA GAT GTT AGT GGA ATG CCA ACA GTT TGC ATT CCG 1634
Val Thr Ile Gly Glu Asp Val Ser Gly Met Pro Thr Val Cys Ile Pro
525 530 535

GTT GAA GAT GGT GGT GTT GGC TTT GAT TAT CGT CTC CAC ATG GCT GTT 1682
Val Glu Asp Gly Gly Val Gly Phe Asp Tyr Arg Leu His Met Ala Val
540 545 550

GCT GAT AAA TGG GTT GAG ATT ATT CAG AAG AGA GAT GAA GAT TGG AAA 1730
Ala Asp Lys Trp Val Glu Ile Ile Gln Lys Arg Asp Glu Asp Trp Lys
555 560 565 570

ATG GGT GAC ATT GTA CAT ATG CTG ACC AAC AGG CGG TGG TTG GAA AAG 1778
Met Gly Asp Ile Val His Met Leu Thr Asn Arg Arg Trp Leu Glu Lys
575 580 585

TGT GTT TCT TAT GCT GAA AGT CAT GAC CAG GCC CTT GTT GGT GAC AAA 1826
Cys Val Ser Tyr Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys
590 595 600

ACT ATT GCA TTT TGG CTG ATG GAC AAG GAT ATG TAT GAC TTC ATG GCT 1874
Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala
605 610 615

CTT GAC AGA CCA TCT ACT CCT CTC ATA GAT CGT GGA GTA GCA TTG CAC 1922
Leu Asp Arg Pro Ser Thr Pro Leu Ile Asp Arg Gly Val Ala Leu His
620 625 630

AAA ATG ATC AGG CTT ATT ACC ATG GGA TTA GGC GGA GAA GGA TAT TTG 1970
Lys Met Ile Arg Leu Ile Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu
635 640 645 650

AAT TTT ATG GGA AAT GAA TTT GGA CAC CCC GAG TGG ATT GAT TTT CCA 2018
Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro
655 660 665

AGA GGT GAT CTA CAT CTT CCC AGT GGT AAA TTT GTT CCT GGG AAC AAT 2066
Arg Gly Asp Leu His Leu Pro Ser Gly Lys Phe Val Pro Gly Asn Asn
670 675 680

TAC AGT TAT GAT AAA TGC CGG CGT AGG TTT GAT CTA GGC AAT TCA AAG 2114
Tyr Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asn Ser Lys
685 690 695

CAT CTG AGA TAT CAT GGA ATG CAA GAG TTT GAT CAA GCA ATT CAG CAT 2162
His Leu Arg Tyr His Gly Met Gln Glu Phe Asp Gln Ala Ile Gln His
700 705 710

CTT GAA GAA GCC TAT GGT TTC ATG ACT TCT GAG CAC CAA TAC ATA TCA 2210
Leu Glu Glu Ala Tyr Gly Phe Met Thr Ser Glu His Gln Tyr Ile Ser
715 720 725 730

CGG AAG GAT GAA AGG GAT CGG ATC ATT GTC TTC GAG AGG GGA AAC CTC 2258
Arg Lys Asp Glu Arg Asp Arg Ile Ile Val Phe Glu Arg Gly Asn Leu
735 740 745

GTT TTT GTA TTC AAT TTT CAT TGG ACT AGC AGC TAT TCG GAT TAC CGA 2306
Val Phe Val Phe Asn Phe His Trp Thr Ser Ser Tyr Ser Asp Tyr Arg
750 755 760

GTT GGC TGC TTA AAG CCA GGA AAG TAC AAG ATA GTC TTG GAT TCA GAT 2354
Val Gly Cys Leu Lys Pro Gly Lys Tyr Lys Ile Val Leu Asp Ser Asp
765 770 775

GAT CCT TTG TTT GGA GGC TTT GGC AGG CTT AGT CAT GAT GCA GAG CAC 2402
Asp Pro Leu Phe Gly Gly Phe Gly Arg Leu Ser His Asp Ala Glu His
780 785 790

TTC AGC TTT GAA GGG TGG TAC GAT AAC CGG CCT CGA TCC TTC ATG GTG 2450
Phe Ser Phe Glu Gly Trp Tyr Asp Asn Arg Pro Arg Ser Phe Met Val
795 800 805 810

TAC ACA CCA TGT AGA ACA GCA GTG GTC TAT GCT TTA GTG GAG GAT GAA 2498
Tyr Thr Pro Cys Arg Thr Ala Val Val Tyr Ala Leu Val Glu Asp Glu
815 820 825

GTG GAG AAT GAA TTG GAA CCT GTC GCC GGT TAA GATATATCTT AACAACAGGT 2551
Val Glu Asn Glu Leu Glu Pro Val Ala Gly *
830 835

TCTGAAGCAG GAATGCCATT ATTGATCTTC CTATGTT 2588

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 837 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Met Gly His Tyr Thr Ile Ser Gly Ile Arg Phe Pro Cys Ala Pro Leu
1 5 10 15

Cys Lys Ser Gln Ser Thr Gly Phe His Gly Tyr Arg Arg Thr Ser Ser
20 25 30

Cys Leu Ser Phe Asn Phe Lys Glu Ala Phe Ser Arg Arg Val Phe Ser
35 40 45

Gly Lys Ser Ser His Glu Ser Asp Ser Ser Asn Val Met Val Thr Ala
50 55 60

Ser Lys Arg Val Leu Pro Asp Gly Arg Ile Glu Cys Tyr Ser Ser Ser
65 70 75 80

Thr Asp Gln Leu Glu Ala Pro Gly Thr Val Ser Glu Glu Ser Gln Val
85 90 95

Leu Thr Asp Val Glu Ser Leu Ile Met Asp Asp Lys Ile Val Glu Asp
100 105 110

Glu Val Asn Lys Glu Ser Val Pro Met Arg Glu Thr Val Ser Ile Arg
115 120 125

Lys Ile Gly Ser Lys Pro Arg Ser Ile Pro Pro Pro Gly Arg Gly Gln
130 135 140

Arg Ile Tyr Asp Ile Asp Pro Ser Leu Thr Gly Phe Arg Gln His Leu
145 150 155 160

Asp Tyr Arg Tyr Ser Gln Tyr Lys Arg Leu Arg Glu Glu Ile Asp Lys
165 170 175

Tyr Glu Gly Ser Leu Asp Ala Phe Ser Arg Gly Tyr Glu Lys Phe Gly
180 185 190

Phe Ser Arg Ser Glu Thr Gly Ile Thr Tyr Arg Glu Trp Ala Pro Gly
195 200 205

Ala Thr Trp Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asn Pro Asn
210 215 220

Ala Asp Val Met Thr Gln Asn Glu Cys Gly Val Trp Glu Ile Phe Leu
225 230 235 240

Pro Asn Asn Ala Asp Gly Ser Pro Pro Ile Pro His Gly Ser Arg Val
245 250 255

Lys Ile Arg Met Asp Thr Pro Ser Gly Asn Lys Asp Ser Ile Pro Ala
260 265 270

Trp Ile Lys Phe Ser Val Gln Ala Pro Gly Glu Leu Pro Tyr Asn Gly
275 280 285

Ile Tyr Tyr Asp Pro Pro Glu Glu Glu Lys Tyr Val Phe Lys Asn Pro
290 295 300

Gln Pro Lys Arg Pro Lys Ser Leu Arg Ile Tyr Glu Ser His Val Gly
305 310 315 320

Met Ser Ser Thr Glu Pro Val Ile Asn Thr Tyr Ala Asn Phe Arg Asp
325 330 335

Asp Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Val Gln Leu
340 345 350

Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr His Val
355 360 365

Thr Asn Phe Tyr Ala Ala Ser Ser Arg Phe Gly Thr Pro Asp Asp Leu
370 375 380

Lys Ser Leu Ile Asp Lys Ala His Glu Leu Gly Leu Leu Val Leu Met
385 390 395 400

Asp Ile Val His Ser His Ala Ser Thr Asn Thr Leu Asp Gly Leu Asn
405 410 415

Met Phe Asp Gly Thr Asp Gly His Tyr Phe His Ser Gly Pro Arg Gly
420 425 430

His His Trp Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Ser Trp Glu
435 440 445

Val Leu Arg Phe Leu Leu Ser Asn Ala Arg Trp Trp Leu Asp Glu Tyr
450 455 460

Lys Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met Tyr Thr
465 470 475 480

His His Gly Leu Gln Val Asp Phe Thr Gly Asn Tyr Asn Glu Tyr Phe
485 490 495

Gly Tyr Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Leu Asn
500 505 510

Asp Met Ile His Gly Leu Phe Pro Glu Ala Val Thr Ile Gly Glu Asp
515 520 525

Val Ser Gly Met Pro Thr Val Cys Ile Pro Val Glu Asp Gly Gly Val
530 535 540

Gly Phe Asp Tyr Arg Leu His Met Ala Val Ala Asp Lys Trp Val Glu
545 550 555 560

Ile Ile Gln Lys Arg Asp Glu Asp Trp Lys Met Gly Asp Ile Val His
565 570 575

Met Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys Val Ser Tyr Ala Glu
580 585 590

Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu
595 600 605

Met Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser Thr
610 615 620

Pro Leu Ile Asp Arg Gly Val Ala Leu His Lys Met Ile Arg Leu Ile
625 630 635 640

Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu
645 650 655

Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg Gly Asp Leu His Leu
660 665 670

Pro Ser Gly Lys Phe Val Pro Gly Asn Asn Tyr Ser Tyr Asp Lys Cys
675 680 685

Arg Arg Arg Phe Asp Leu Gly Asn Ser Lys His Leu Arg Tyr His Gly
690 695 700

Met Gln Glu Phe Asp Gln Ala Ile Gln His Leu Glu Glu Ala Tyr Gly
705 710 715 720

Phe Met Thr Ser Glu His Gln Tyr Ile Ser Arg Lys Asp Glu Arg Asp
725 730 735

Arg Ile Ile Val Phe Glu Arg Gly Asn Leu Val Phe Val Phe Asn Phe
740 745 750

His Trp Thr Ser Ser Tyr Ser Asp Tyr Arg Val Gly Cys Leu Lys Pro
755 760 765

Gly Lys Tyr Lys Ile Val Leu Asp Ser Asp Asp Pro Leu Phe Gly Gly
770 775 780

Phe Gly Arg Leu Ser His Asp Ala Glu His Phe Ser Phe Glu Gly Trp
785 790 795 800

Tyr Asp Asn Arg Pro Arg Ser Phe Met Val Tyr Thr Pro Cys Arg Thr
805 810 815

Ala Val Val Tyr Ala Leu Val Glu Asp Glu Val Glu Asn Glu Leu Glu
820 825 830

Pro Val Ala Gly *
835

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2805 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:131..2677

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

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AGTGAATTCG AGCTCGGTAC CCGGGGATCC GATTCGCATT TCTCGCTATT GCTTTCCGTT     60

TATTTCCATA TATAAAATAT CAAATCTAAT CACTTGCGCC ATTTCTATCT CTCTCCAAAC    120

TCTCACCGAA ATG GTA TAC TAC ACT GTA TCA GGC ATA CGT TTT CCT TGT       169
           Met Val Tyr Tyr Thr Val Ser Gly Ile Arg Phe Pro Cys
                   840                 845                 850

GCA CCT TCA CTC TAC AAA TCT CAG CTC ACC AGC TTC CAT GGC GGT CGA      217
Ala Pro Ser Leu Tyr Lys Ser Gln Leu Thr Ser Phe His Gly Gly Arg
                855                 860                 865

AGG ACC TCT TCT GGC CTT TCC TTC CTC TTG AAG AAG GAG CTG TTT CCT      265
Arg Thr Ser Ser Gly Leu Ser Phe Leu Leu Lys Lys Glu Leu Phe Pro
            870                 875                 880

CGG AAG ATC TTT GCT GGA AAG TCC TCT TAT GAA TCT GAC TCC TCA AAT      313
Arg Lys Ile Phe Ala Gly Lys Ser Ser Tyr Glu Ser Asp Ser Ser Asn
        885                 890                 895

TTA ACT GTC TCT GCA TCT GAG AAG GTC CTT GTT CCT GAT GAT CAG ATT      361
Leu Thr Val Ser Ala Ser Glu Lys Val Leu Val Pro Asp Asp Gln Ile
    900                 905                 910

GAT GGC TCT TCT TCT TCA ACA TAT CAA TTA GAA ACC ACT GGC ACA GTT      409
Asp Gly Ser Ser Ser Ser Thr Tyr Gln Leu Glu Thr Thr Gly Thr Val
915                 920                 925                 930

TTG GAG GAA TCC CAG GTT CTT GGT GAT GCA GAG AGT CTT GTG ATG GAA      457
Leu Glu Glu Ser Gln Val Leu Gly Asp Ala Glu Ser Leu Val Met Glu
                935                 940                 945

GAT GAT AAG AAT GTT GAG GAG GAT GAA GTA AAA AAA GAG TCG GTT CCA      505
Asp Asp Lys Asn Val Glu Glu Asp Glu Val Lys Lys Glu Ser Val Pro
            950                 955                 960

TTG CAT GAG ACA ATT AGC ATT GGA AAA AGT GAA TCT AAA CCA AGG TCC      553
Leu His Glu Thr Ile Ser Ile Gly Lys Ser Glu Ser Lys Pro Arg Ser
        965                 970                 975

ATT CCT CCA CCT GGC AGT GGG CAG AGA ATA TAT GAC ATA GAT CCA AGC      601
Ile Pro Pro Pro Gly Ser Gly Gln Arg Ile Tyr Asp Ile Asp Pro Ser
    980                 985                 990

TTG GCA GGT TTC CGT CAG CAT CTT GAC TAC CGA TAT TCA CAG TAC AAA      649
Leu Ala Gly Phe Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys
995                1000                1005                1010

AGG CTG CGT GAG GAA ATT GAC AAG TAT GAA GGT GGT TTG GAT GCA TTC      697
Arg Leu Arg Glu Glu Ile Asp Lys Tyr Glu Gly Gly Leu Asp Ala Phe
               1015                1020                1025

TCT CGT GGA TTT GAA AAG TTT GGT TTC TTA CGC AGT GAA ACA GGA ATA      745
Ser Arg Gly Phe Glu Lys Phe Gly Phe Leu Arg Ser Glu Thr Gly Ile
           1030                1035                1040
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ACT TAT AGG GAA TGG GCA CCT GGA GCT ACG TGG GCT GCA CTT ATT GGA 793
Thr Tyr Arg Glu Trp Ala Pro Gly Ala Thr Trp Ala Ala Leu Ile Gly
1045 1050 1055

GAT TTC AAC AAT TGG AAT CCT AAT GCA GAT GTC ATG ACT CGG AAT GAG 841
Asp Phe Asn Asn Trp Asn Pro Asn Ala Asp Val Met Thr Arg Asn Glu
1060 1065 1070

TTT GGT GTC TGG GAG ATT TTT TTG CCA AAT AAC GCA GAT GGT TCA CCA 889
Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Ser Pro
1075 1080 1085 1090

CCA ATT CCT CAT GGT TCT CGA GTA AAG ATA CGC ATG GAT ACT CCA TCT 937
Pro Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro Ser
1095 1100 1105

GGC ATC AAA GAT TCA ATT CCT GCT TGG ATC AAG TTC TCA GTT CAG GCA 985
Gly Ile Lys Asp Ser Ile Pro Ala Trp Ile Lys Phe Ser Val Gln Ala
1110 1115 1120

CCT GGT GAA ATC CCA TAC AAT GCC ATA TAC TAT GAT CCA CCA AAG GAG 1033
Pro Gly Glu Ile Pro Tyr Asn Ala Ile Tyr Tyr Asp Pro Pro Lys Glu
1125 1130 1135

GAG AAG TAT GTG TTC AAA CAT CCT CAG CCA AAG-AGA CCA AAA TCA CTT 1081
Glu Lys Tyr Val Phe Lys His Pro Gln Pro Lys Arg Pro Lys Ser Leu
1140 1145 1150

AGG ATT TAT GAA TCT CAT GTT GGG ATG AGT AGT ATG GAG CCA ATA ATT 1129
Arg Ile Tyr Glu Ser His Val Gly Met Ser Ser Met Glu Pro Ile Ile
1155 1160 1165 1170

AAC ACA TAT GCC AAC TTT AGA GAT GAT ATG CTT CCT CGC ATC AAA AAG 1177
Asn Thr Tyr Ala Asn Phe Arg Asp Asp Met Leu Pro Arg Ile Lys Lys
1175 1180 1185

CTT GGC TAC AAT GCT GTT CAG ATC ATG GCT ATT CAA GAG CAT TCC TAT 1225
Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln Glu His Ser Tyr
1190 1195 1200

TAT GCT AGT TTT GGG TAC CAT GTC ACA AAC TTT TTT GCA CCT AGC AGC 1273
Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser Ser
1205 1210 1215

CGA TTT GGA ACT CCT GAT GAT TTG AAG TCT TTA ATA GAT AAA GCT CAT 1321
Arg Phe Gly Thr Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala His
1220 1225 1230

GAG TTA GGG CTG CTT GTT CTC ATG GAT ATT GTT CAT AGC CAT GCG TCA 1369
Glu Leu Gly Leu Leu Val Leu Met Asp Ile Val His Ser His Ala Ser
1235 1240 1245 1250

AAT AAT ACG TTG GAT GGG CTG AAC ATG TTT GAT GGT ACG GAT AGT CAC 1417
Asn Asn Thr Leu Asp Gly Leu Asn Met Phe Asp Gly Thr Asp Ser His
1255 1260 1265

TAC TTC CAC TCC GGA TCA CGG GGT CAT CAT TGG TTG TGG GAC TCT CGC 1465
Tyr Phe His Ser Gly Ser Arg Gly His His Trp Leu Trp Asp Ser Arg
1270 1275 1280

CTT TTC AAC TAT GGA AGC TGG GAG GTG CTA AGA TTT CTT CTT TCA AAT 1513
Leu Phe Asn Tyr Gly Ser Trp Glu Val Leu Arg Phe Leu Leu Ser Asn
1285 1290 1295

GCA AGA TGG TGG TTG GAA GAG TAC AGG TTT GAT GGT TTT AGA TTT GAT 1561
Ala Arg Trp Trp Leu Glu Glu Tyr Arg Phe Asp Gly Phe Arg Phe Asp
1300 1305 1310

GGG GTG ACT TCC ATG ATG TAC ACT CCC CAT GGG TTG CAG GTA GCT TTT 1609
Gly Val Thr Ser Met Met Tyr Thr Pro His Gly Leu Gln Val Ala Phe
1315 1320 1325 1330

ACT GGC AAC TAC AAT GAG TAC TTT GGA TAT GCA ACT GAT GTA GAT GCT 1657
Thr Gly Asn Tyr Asn Glu Tyr Phe Gly Tyr Ala Thr Asp Val Asp Ala
1335 1340 1345

GTG ATT TAT TTG ATG CTT GTG AAT GAT ATG ATT CAC GGT CTT TTC CCT 1705
Val Ile Tyr Leu Met Leu Val Asn Asp Met Ile His Gly Leu Phe Pro
1350 1355 1360

GAG GCT GTT ACC ATT GGT GAA GAT GTT AGC GGA AAG CCA ACA TTT TGC 1753
Glu Ala Val Thr Ile Gly Glu Asp Val Ser Gly Lys Pro Thr Phe Cys
1365 1370 1375

ATT CCA GTG GAA GAT GGT GGT GTT GGA TTT GAT TAC CGT CTC CAC ATG 1801
Ile Pro Val Glu Asp Gly Gly Val Gly Phe Asp Tyr Arg Leu His Met
1380 1385 1390

GCC ATT GCC GAT AAA TGG ATT GAG ATT CTT AAG AAG AGA GAT GAG GAC 1849
Ala Ile Ala Asp Lys Trp Ile Glu Ile Leu Lys Lys Arg Asp Glu Asp
1395 1400 1405 1410

TGG AAA ATG GGT GAC ATT GTG CAT ACA CTC ACC AAC AGA AGG TGG TTG 1897
Trp Lys Met Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp Leu
1415 1420 1425

GAA AAA TGT GTT GCT TAT GCT GAA AGT CAT GAC CAA GCT CTT GTT GGT 1945
Glu Lys Cys Val Ala Tyr Ala Glu Ser His Asp Gln Ala Leu Val Gly
1430 1435 1440

GAC AAA ACT ATT GCA TTT TGG CTG ATG GAC AAG GAC ATG TAC GAC TTC 1993
Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp Phe
1445 1450 1455

ATG GCT CGT GAC AGA CCA TCT ACT CCT CTT ATA GAT CGT GGA ATA GCA 2041
Met Ala Arg Asp Arg Pro Ser Thr Pro Leu Ile Asp Arg Gly Ile Ala
1460 1465 1470

TTG CAC AAA ATG ATC AGG CTT ATT ACC ATG GGC TTA GGC GGA GAA GGA 2089
Leu His Lys Met Ile Arg Leu Ile Thr Met Gly Leu Gly Gly Glu Gly
1475 1480 1485 1490

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TAT TTG AAT TTT ATG GGA AAT GAA TTT GGA CAT CCT GAG TGG ATT GAT     2137
Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp
                1495                1500                1505

TTT CCA AGA GGG GAT CGA CAT CTG CCC AAT GGT AAA GTA ATT CCA GGG     2185
Phe Pro Arg Gly Asp Arg His Leu Pro Asn Gly Lys Val Ile Pro Gly
            1510                1515                1520

AAC AAC CAC AGT TAT GAT AAA TGC CGT CGT AGA TTT GAT CTA GGT GAT     2233
Asn Asn His Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp
        1525                1530                1535

GCA GAC TAT CTA AGA TAT CAT GGA ATG CAA GAG TTT GAT CAG GCA ATG     2281
Ala Asp Tyr Leu Arg Tyr His Gly Met Gln Glu Phe Asp Gln Ala Met
    1540                1545                1550

CAA CAT CTT GAA GAA GCC TAT GGT TTC ATG ACT TCT GAG CAC CAG TAT     2329
Gln His Leu Glu Glu Ala Tyr Gly Phe Met Thr Ser Glu His Gln Tyr
1555                1560                1565                1570

ATA TCA CGG AAG GAT GAA GGA GAT CGG ATC ATT GTC TTT GAG AGG GGA     2377
Ile Ser Arg Lys Asp Glu Gly Asp Arg Ile Ile Val Phe Glu Arg Gly
                1575                1580                1585

AAC CTT GTT TTT GTA TTC AAC TTT CAT TGG ACT AAC AGC TAT TCA GAT     2425
Asn Leu Val Phe Val Phe Asn Phe His Trp Thr Asn Ser Tyr Ser Asp
            1590                1595                1600

TAC CGA GTT GGC TGC TTC AAG TCA GGA AAG TAC AAG ATT GTT TTG GAC     2473
Tyr Arg Val Gly Cys Phe Lys Ser Gly Lys Tyr Lys Ile Val Leu Asp
        1605                1610                1615

TCG GAT GAT GGC TTG TTT GGA GGC TTC AAC AGG CTT AGT CAT GAT GCC     2521
Ser Asp Asp Gly Leu Phe Gly Gly Phe Asn Arg Leu Ser His Asp Ala
    1620                1625                1630

GAG CAC TTC ACC TTT GAC GGG TGG TAT GAT AAC CGG CCT CGG TCC TTC     2569
Glu His Phe Thr Phe Asp Gly Trp Tyr Asp Asn Arg Pro Arg Ser Phe
1635                1640                1645                1650

ATG GTA TAT GCA CCA TCT AGG ACA GCA GTG GTC TAT GCT TTA GTA GAA     2617
Met Val Tyr Ala Pro Ser Arg Thr Ala Val Val Tyr Ala Leu Val Glu
                1655                1660                1665

GAT GAA GAG AAT GAA GCA GAG AAT GAA GTA GAA AGT GAA GTG AAA CCA     2665
Asp Glu Glu Asn Glu Ala Glu Asn Glu Val Glu Ser Glu Val Lys Pro
            1670                1675                1680

GCC TCC GGC TGA GATAGATATT TAGTAAGAGG ATCCCCTAAA GCAGGAATGG          2717
Ala Ser Gly  *
        1685

TTAACCTGTG CATCTGCATT GAACGACGTA TATTGAGACT GGAAATCCAT ATGACTAGTA   2777

GATCCTCTAG AGTCGACCTG CAGGCATG                                      2805
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(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 849 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

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Met Val Tyr Tyr Thr Val Ser Gly Ile Arg Phe Pro Cys Ala Pro Ser
 1               5                  10                  15

Leu Tyr Lys Ser Gln Leu Thr Ser Phe His Gly Gly Arg Arg Thr Ser
            20                  25                  30

Ser Gly Leu Ser Phe Leu Leu Lys Lys Glu Leu Phe Pro Arg Lys Ile
        35                  40                  45

Phe Ala Gly Lys Ser Ser Tyr Glu Ser Asp Ser Ser Asn Leu Thr Val
    50                  55                  60

Ser Ala Ser Glu Lys Val Leu Val Pro Asp Asp Gln Ile Asp Gly Ser
65                  70                  75                  80

Ser Ser Ser Thr Tyr Gln Leu Glu Thr Thr Gly Thr Val Leu Glu Glu
                85                  90                  95

Ser Gln Val Leu Gly Asp Ala Glu Ser Leu Val Met Glu Asp Asp Lys
            100                 105                 110

Asn Val Glu Glu Asp Glu Val Lys Lys Glu Ser Val Pro Leu His Glu
        115                 120                 125

Thr Ile Ser Ile Gly Lys Ser Glu Ser Lys Pro Arg Ser Ile Pro Pro
    130                 135                 140

Pro Gly Ser Gly Gln Arg Ile Tyr Asp Ile Asp Pro Ser Leu Ala Gly
145                 150                 155                 160

Phe Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Arg Leu Arg
                165                 170                 175

Glu Glu Ile Asp Lys Tyr Glu Gly Gly Leu Asp Ala Phe Ser Arg Gly
            180                 185                 190

Phe Glu Lys Phe Gly Phe Leu Arg Ser Glu Thr Gly Ile Thr Tyr Arg
        195                 200                 205

Glu Trp Ala Pro Gly Ala Thr Trp Ala Ala Leu Ile Gly Asp Phe Asn
    210                 215                 220

Asn Trp Asn Pro Asn Ala Asp Val Met Thr Arg Asn Glu Phe Gly Val
225                 230                 235                 240
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Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Ser Pro Pro Ile Pro
                245                 250                 255

His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Ile Lys
            260                 265                 270

Asp Ser Ile Pro Ala Trp Ile Lys Phe Ser Val Gln Ala Pro Gly Glu
        275                 280                 285

Ile Pro Tyr Asn Ala Ile Tyr Tyr Asp Pro Pro Lys Glu Glu Lys Tyr
    290                 295                 300

Val Phe Lys His Pro Gln Pro Lys Arg Pro Lys Ser Leu Arg Ile Tyr
305                 310                 315                 320

Glu Ser His Val Gly Met Ser Ser Met Glu Pro Ile Ile Asn Thr Tyr
                325                 330                 335

Ala Asn Phe Arg Asp Asp Met Leu Pro Arg Ile Lys Lys Leu Gly Tyr
            340                 345                 350

Asn Ala Val Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser
        355                 360                 365

Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly
    370                 375                 380

Thr Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala His Glu Leu Gly
385                 390                 395                 400

Leu Leu Val Leu Met Asp Ile Val His Ser His Ala Ser Asn Asn Thr
                405                 410                 415

Leu Asp Gly Leu Asn Met Phe Asp Gly Thr Asp Ser His Tyr Phe His
            420                 425                 430

Ser Gly Ser Arg Gly His His Trp Leu Trp Asp Ser Arg Leu Phe Asn
        435                 440                 445

Tyr Gly Ser Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Ala Arg Trp
    450                 455                 460

Trp Leu Glu Glu Tyr Arg Phe Asp Gly Phe Arg Phe Asp Gly Val Thr
465                 470                 475                 480

Ser Met Met Tyr Thr Pro His Gly Leu Gln Val Ala Phe Thr Gly Asn
                485                 490                 495

Tyr Asn Glu Tyr Phe Gly Tyr Ala Thr Asp Val Asp Ala Val Ile Tyr
            500                 505                 510

Leu Met Leu Val Asn Asp Met Ile His Gly Leu Phe Pro Glu Ala Val
        515                 520                 525

Thr Ile Gly Glu Asp Val Ser Gly Lys Pro Thr Phe Cys Ile Pro Val
    530                 535                 540
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Glu Asp Gly Gly Val Gly Phe Asp Tyr Arg Leu His Met Ala Ile Ala
545 550 555 560

Asp Lys Trp Ile Glu Ile Leu Lys Lys Arg Asp Glu Asp Trp Lys Met
565 570 575

Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp Leu Glu Lys Cys
580 585 590

Val Ala Tyr Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr
595 600 605

Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala Arg
610 615 620

Asp Arg Pro Ser Thr Pro Leu Ile Asp Arg Gly Ile Ala Leu His Lys
625 630 635 640

Met Ile Arg Leu Ile Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn
645 650 655

Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg
660 665 670

Gly Asp Arg His Leu Pro Asn Gly Lys Val Ile Pro Gly Asn Asn His
675 680 685

Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Asp Tyr
690 695 700

Leu Arg Tyr His Gly Met Gln Glu Phe Asp Gln Ala Met Gln His Leu
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Glu Glu Ala Tyr Gly Phe Met Thr Ser Glu His Gln Tyr Ile Ser Arg
725 730 735

Lys Asp Glu Gly Asp Arg Ile Ile Val Phe Glu Arg Gly Asn Leu Val
740 745 750

Phe Val Phe Asn Phe His Trp Thr Asn Ser Tyr Ser Asp Tyr Arg Val
755 760 765

Gly Cys Phe Lys Ser Gly Lys Tyr Lys Ile Val Leu Asp Ser Asp Asp
770 775 780

Gly Leu Phe Gly Gly Phe Asn Arg Leu Ser His Asp Ala Glu His Phe
785 790 795 800

Thr Phe Asp Gly Trp Tyr Asp Asn Arg Pro Arg Ser Phe Met Val Tyr
805 810 815

Ala Pro Ser Arg Thr Ala Val Val Tyr Ala Leu Val Glu Asp Glu Glu
820 825 830

Asn Glu Ala Glu Asn Glu Val Glu Ser Glu Val Lys Pro Ala Ser Gly
835 840 845

CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nucleic acid sequence encoding a polypeptide, the encoded polypeptide comprising at least a portion of the amino acid sequence shown in Figure 4 or Figure 13 which retains sufficient starch branching enzyme (SBE) activity when expressed in E. coli KV 832 to complement the branching enzyme mutation therein; at least 200 bp; and exhibiting at least 88% sequence identity with the corresponding region of the DNA sequence shown in Figures 4, 9, 10 or 13, operably linked in the sense or anti-sense orientation to a promoter operable in plants.

2. A nucleic acid sequence according to claim 1, comprising nucleotides 21-2531 of the nucleic acid sequence shown in Figure 4, or a functionally equivalent nucleotide sequence which hybridises under stringent hybridisation conditions with the nucleic acid sequence shown in Figure 4.

3. A nucleic acid sequence according to claim 1, comprising nucleotides 131-2677 of the nucleic acid sequence shown in Figure 13, or a functionally equivalent sequence which hybridises under stringent hybridisation conditions with the nucleic acid sequence shown in Figure 13.

4. A nucleic acid sequence according to any one of claims 1, 2 or 3 comprising a 5' and/or a 3' untranslated region.

5. A nucleic acid sequence according to any one of claims 1 o 4, encoding a polypeptide having the amino acid sequence NSKH at about residue 697.

6. A nucleic acid sequence comprising at least 200 bp and exhibiting at least 88% sequence identity with the corresponding region of the DNA sequence shown in Figures 4, 9, 10 or 13, operably linked in the sense or anti-sense orientation to a promoter operable in plants.

7. A nucleic acid sequence according to claim 6, comprising at least 300-600bp.

8. A sequence according to claim 6 or 7, comprising a 5' and/or 3' untranslated region.

9. A sequence according to claim 8, comprising nucleotides 688-1044 of the sequence shown in figure 9, and/or nucleotides 1507-1900 of the sequence shown in Figure 10.

10. A sequence according to claim 6, comprising the nucleotide sequence shown in Figure 10.

11. A replicable nucleic acid construct comprising a nucleic acid sequence according to any one of the preceding claims.

12. A polypeptide comprising at least a portion of the amino acid sequence shown in Figure 4 or Figure 13 which retains sufficient starch branching enzyme (SBE) activity when expressed in E. coli KV 832 to complement the branching enzyme mutation therein; encoded by a nucleic acid with at least 200 bp which exhibits at least 88% sequence identity with the corresponding region of the DNA sequence shown in Figures 4, 9, 10 or 13, operably linked in the sense or anti-sense orientation to a promoter operable in plants.

13. A polypeptide according to claim 12, in substantial isolation from other polypeptides.

14. A polypeptide according to claim 12 or 13, having the amino acid sequence NSKH at about position 697.

15. A method of modifying starch *in vitro*, the method comprising treating starch to be modified under suitable conditions with an effective amount of a polypeptide according to any one of claims 12, 13 or 14.

16. A method of altering a plant host cell, the method comprising introducing into the cell a nucleic acid sequence comprising at least 200 bp and exhibiting at least 88% sequence identity with the corresponding region of the DNA sequence shown in figures 4, 9, 10 or 13, operably linked in the sense or anti-sense orientation to a suitable promoter active in the host cell, and causing transcription of

the introduced nucleotide sequence, said transcript and/or the translation product thereof being sufficient to interfere with the expression of a homologous gene naturally present in the host cell, which homologous gene encodes a polypeptide having SBE activity.

17. A method according to claim 16, wherein the host cell is from a cassava, banana, potato, pea, tomato, maize, wheat, barley, oat, sweet potato or rice plant.

18. A method according to claim 16 or 17, comprising the introduction of one or more further nucleic acid sequences, operably linked in the sense or anti-sense orientation to a suitable promoter active in the host cell, and causing transcription of the one or more further nucleic acid sequences, said transcripts and/or translation products thereof being sufficient to interfere with the expression of homologous gene(s) present in the host cell.

19. A method according to claim 18, wherein the one or more further nucleic acid sequences interfere with the expression of a gene involved in starch biosynthesis.

20. A method according to claim 18 or 19, wherein the further nucleic acid sequence comprises at least part of an SBE I gene.

21. A method according to claim 20, wherein the further nucleic acid sequence comprises at least part of the cassava SBE I gene.

22. A method according to any one of claims 16-21, wherein the host cell is selected from one of the following: cassava, banana, potato, pea, tomato, maize, wheat, barley, oat, sweet potato or rice.

23. A method according to any one of claims 16-22, wherein the altered host cell gives rise to starch having different properties compared to starch from an unaltered cell.

24. A method according to any one of claims 16-23, further comprising the step of growing the altered host cell into a plant or plantlet.

25. A method of obtaining starch having altered properties, comprising growing a plant from an altered host cell according to the method of claim 24, and extracting the starch therefrom.

26. A plant or plant cell into which has been artificially introduced a nucleic acid sequence comprising at least 200bp and exhibiting at least 88% sequence identity with the corresponding region of the DNA sequence shown in Figures 4, 9, 10, or 13, operably linked in the sense or anti-sense orientation to a promoter operable in plants, or the progeny thereof.

27. A plant according to claim 24, altered by the method of any one of claims 16-22.

28. Starch obtainable from an altered plant according to claim 26 or 27, having altered properties compared to starch extracted from an equivalent but unaltered plant.

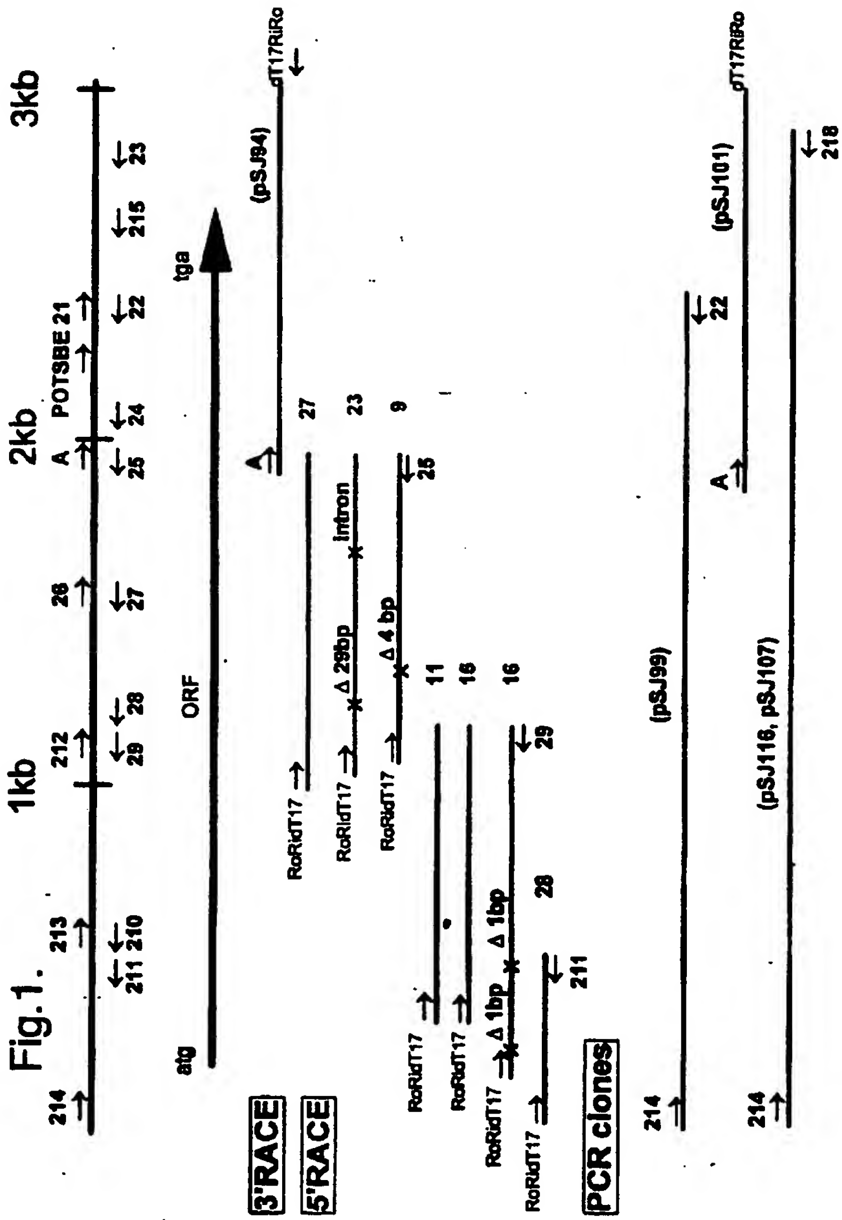
29. Starch obtained from an altered plant according to claim 26 or 27, having altered properties compared to starch extracted from an equivalent but unaltered plant.

30. Starch according to claim 28 or 29 obtained from an altered plant selected from the group consisting of: cassava, banana, potato, pea, tomato, maize, wheat, barley, oat, sweet potato and rice plants.

31. Starch according to any one of claims 28, 29 or 30, having increased amylose content compared to starch extracted from an equivalent but unaltered plant.

Dated this 21st day of December 2000

NATIONAL STARCH AND CHEMICAL INVESTMENT HOLDING CORPORATION
By their Patent Attorneys
COLLISON & CO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5'RACE
(pSJ94)
dT17RiRo
RoRidT17
Δ 29bp
Δ 4 bp
intron
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Fig.1.

Fig.2.

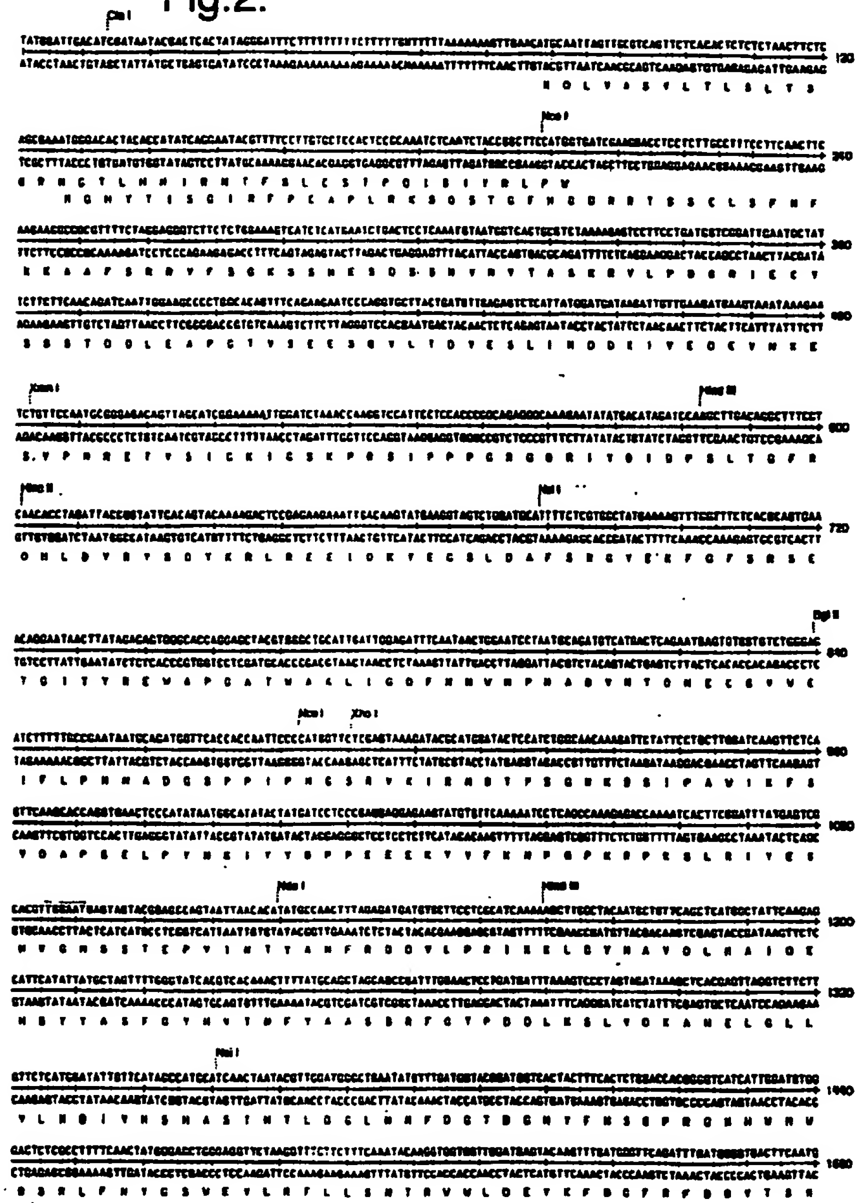
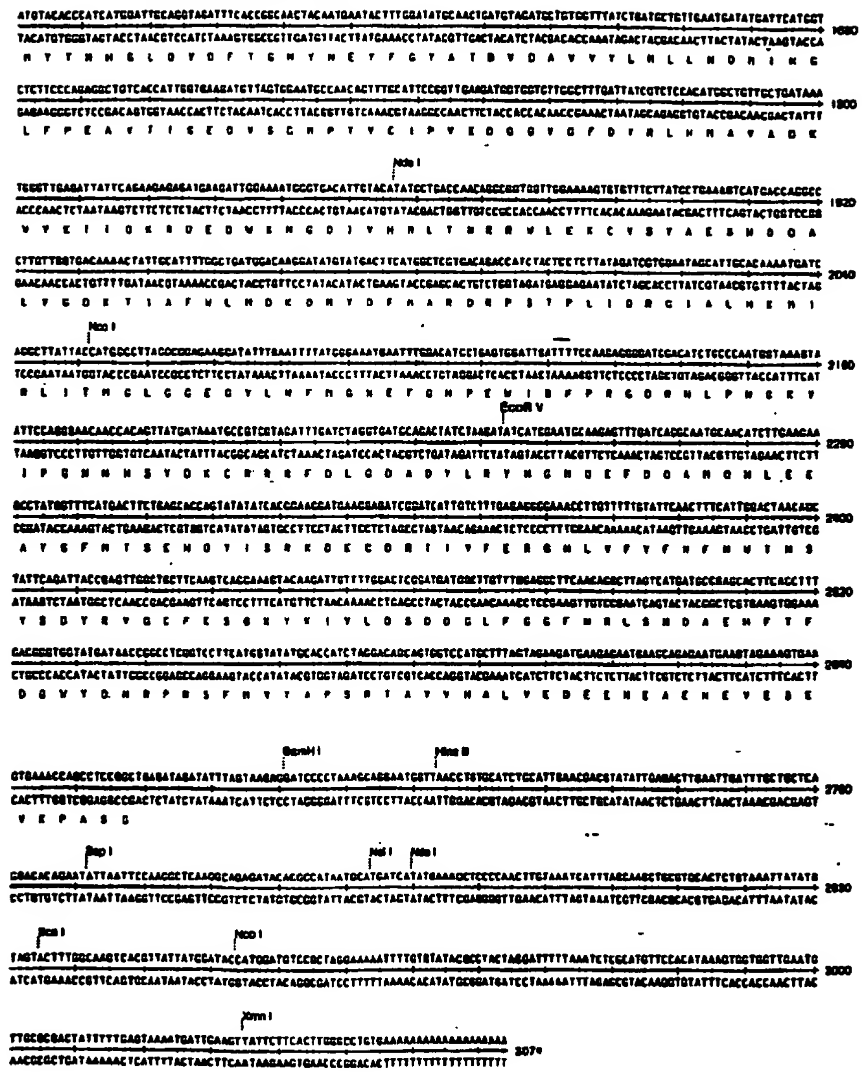


Fig.2 (Cont).



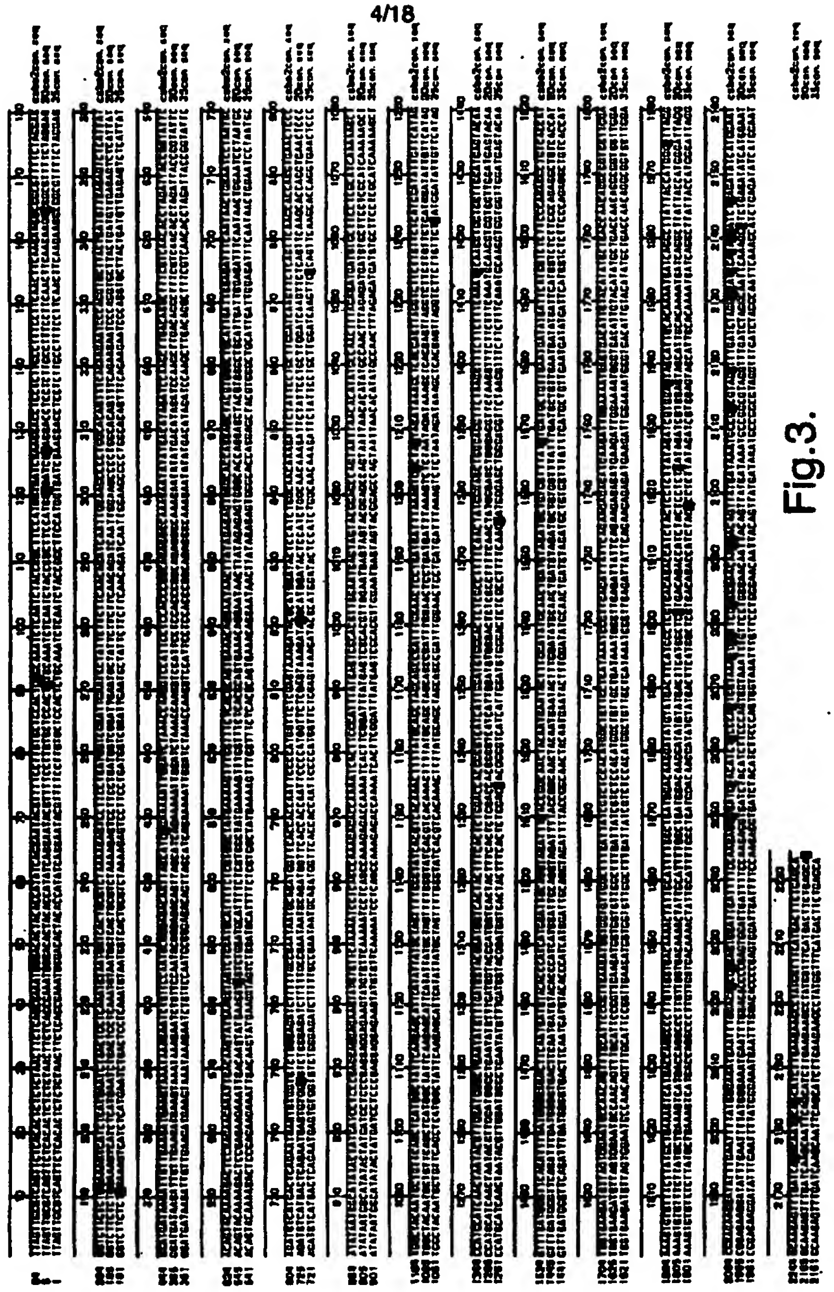


Fig.3.

Fig.4.

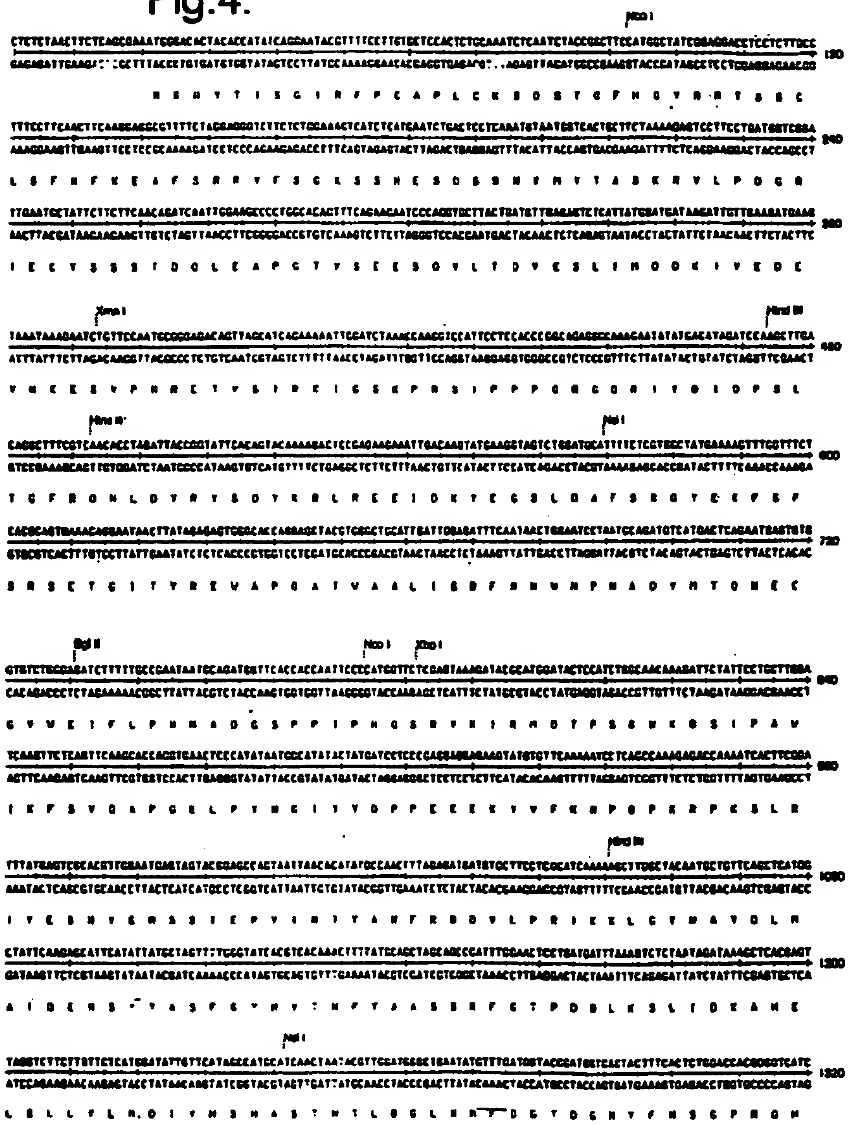
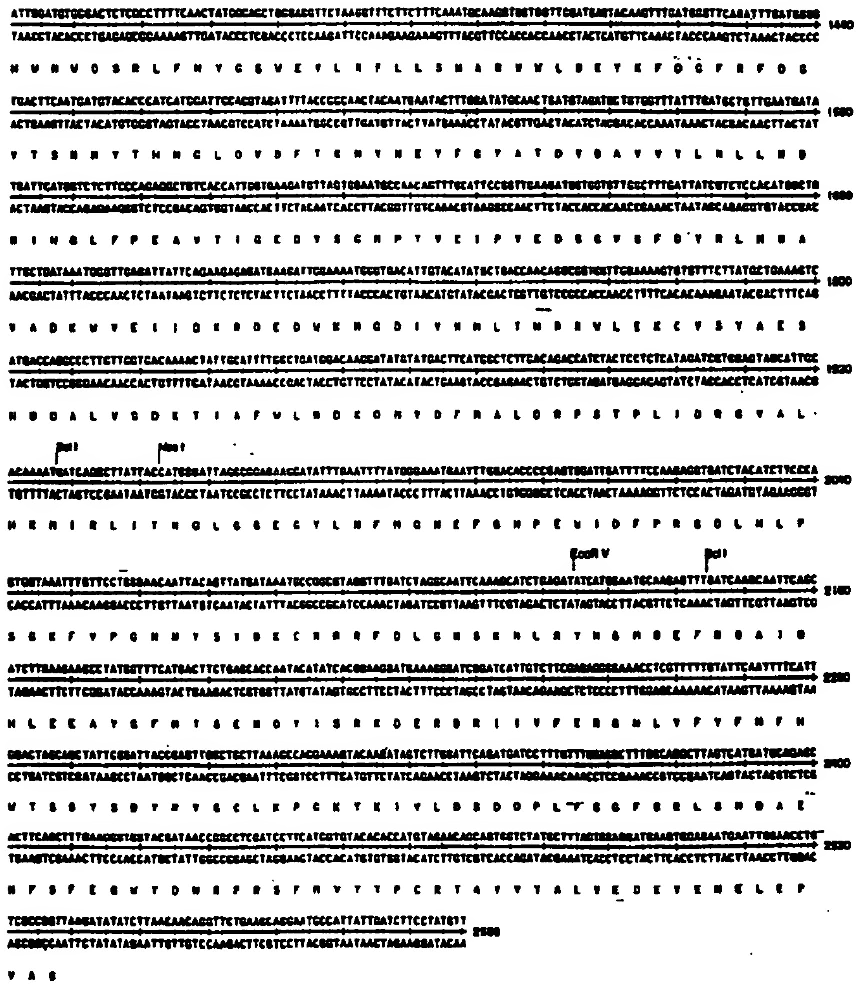


Fig.4 (Cont).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1560
1680
1800
1920
Bcl I
Nco I
2040
EcoR V
Bcl I
2160
2280
2400
2520

Fig.5.

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              60        70        80        90        100       110       120
125-94. seq   TAGTTTTGGGTACCATGTCACAAACTTTTTTGCACCTAGCAGCCGATTTGGAACTCCTGATGATTTGAAG
              TAGTTTTGGGTA CA GTCACAAACTTTT TGCA CTAGCAGCCGATTTGGAACTCCTGATGATTT AAG
116. seq      TAGTTTTGGGTATCACGTCACAAACTTTTATGCAGCTAGCAGCCGATTTGGAACTCCTGATGATTTAAAG
              1140      1150      1160      1170      1180      1190      1200
              130       140       150       160       170       180       190
125-94. seq   TCTTTAATAGATAAAGCTCATGAGTTAGGGCTGCTTGTTCTCATGGATATTGTTCATAGCCATGCGTCAA
              TCT TAATAGATAAAGCTCA GAGTTAGG CT CTTGTTCTCATGGATATTGTTCATAGCCATGC TCAA
116. seq      TCTCTAATAGATAAAGCTCACGAGTTAGGTCTTCTTGTTCTCATGGATATTGTTCATAGCCATGCATCAA
              1210      1220      1230      1240      1250      1260      1270
              200       210       220       230       240       250       260
125-94. seq   ATAATACGTTGGATGGGCTGAACATGTTTGATGGTACGGATAGTCACTACTTCCACTCCGGATCACGGGG
               TAATACGTTGGATGGGCTGAA ATGTTTGATGGTACGGAT GTCACTACTT CACTC GGA CACGGGG
116. seq      CTAATACGTTGGATGGGCTGAATATGTTTGATGGTACGGATGGTCACTACTTTCACTCTGGACCACGGGG
              1280      1290      1300      1310      1320      1330      1340
              270       280       290       300       310       320       330
125-94. seq   TCATCATTGGTTGTGGGACTCTCGCCTTTTCAACTATGGAAGCTGGGAGGTGCTAAGATTTCTTCTTTCA
              TCATCATTGG TGTGGGACTC CGCCTTTTCAACTATGG AGCTGGGAGGT CTAAG TTTCTTCTTTCA
116. seq      TCATCATTGGATGTGGGACTCCCGCCTTTTCAACTATGGGAGCTGGGAGGTTCTAAGGTTTCTTCTTTCA
              1350      1360      1370      1380      1390      1400      1410
              340       350       360       370       380       390       400
125-94. seq   AATGCAAGATGGTGGTTGGAAGAGTACAGGTTTGATGGTTTTAGATTTGATGGGGTGACTTCCATGATGT
              AATGCAAG TGGTGGTTGGA GAGTACA GTTTGATGG TT AGATTTGA GGGGTGACTTC ATGATGT
116. seq      AATGCAAGGTGGTGGTTGGATGAGTACAAGTTTGATGGGTTCAGATTTGACGGGGTGACTTCAATGATGT
              1420      1430      1440      1450      1460      1470      1480
              410       420       430       440       450       460       470
125-94. seq   ACACTCCCCATGGGTTGCAGGTAGCTTTTACTGGCAACTACAATGAGTACTTTGGATATGCAACTGATGT
              ACAC C  CATGG TTGCAGGTAG TTTTAC GGCAACTACAATGA TACTTTGGATATGCAACTGATGT
116. seq      ACACCCATCATGGATTGCAGGTAGATTTTACCGGCAACTACAATGAATACTTTGGATATGCAACTGATGT
              1490      1500      1510      1520      1530      1540      1550
              480       490       500       510       520       530       540
125-94. seq   AGATGCTGTGATTTATTTGATGCTTGTGAATGATATGATTCACGGTCTTTTCCCTGAGGCTGTTACCATT
              AGATGCTGTG TTTATTTGATGCT  TGAATGATATGATTCA GGTCT TTCCC GAGGCTGT ACCATT
116. seq      AGATGCTGTGGTTTATTTGATGCTGTTGAATGATATGATTCATGGTCTCTTCCCAGAGGCTGTCACCATT
              1560      1570      1580      1590      1600      1610      1620
              550       560       570       580       590       600       610
125-94. seq   GGTGAAGATGTTAGCGGAAAGCCAACATTTTGCATTCCAGTGGAAGATGGTGGTGTTGGATTTGATTACC
              GGTGAAGATGTTAG GGAA GCCAACA TTTGCATTCC GT GAAGATGGTGGTGTTGG TTTGATTA C
116. seq      GGTGAAGATGTTAGTGGAATGCCAACAGTTTGCATTCCGGTTGAAGATGGTGGTGTTGGCTTTGATTATC
              1630      1640      1650      1660      1670      1680      1690
              620       630       640       650       660       670       680
125-94. seq   GTCTCCACATGGCCATTGCCGATAAATGGATTGAGATTCTTAAGAAGAGAGATGAGGACTGGAAAATGGG
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116. seq      GTCTCCACATGGCTGTTGCTGATAAATGGGTTGAGATTATTCAGAAGAGAGATGAAGATTGGAAAATGGG
              1700      1710      1720      1730      1740      1750      1760
              690       700       710       720       730       740       750
125-94. seq   TGACATTGTGCATACACTCACCAACAGAAGGTGGTTGGAAAAATGTGTTGCTTATGCTGAAAGTCATGAC
              TGACATTGT CATA  CT ACCAACAG  GGTGGTTGGAAAA TGTGTT CTTATGCTGAAAGTCATGAC
116. seq      TGACATTGTACATATGCTGACCAACAGGCGGTGGTTGGAAAAGTGTGTTTCTTATGCTGAAAGTCATGAC
              1770      1780      1790      1800      1810      1820      1830
              760       770       780       790       800       810       820
125-94. seq   CAAGCTCTTGTTGGTGACAAAACTATTGCATTTTGGCTGATGGACAAGGACATGTACGACTTCATGGCTC
              CA GC CTTGTTGGTGACAAAACTATTGCATTTTGGCTGATGGACAAGGA ATGTA GACTTCATGGCTC
116. seq      CAGGCCCTTGTTGGTGACAAAACTATTGCATTTTGGCTGATGGACAAGGATATGTATGACTTCATGGCTC
              1840      1850      1860      1870      1880      1890      1900
              830       840       850       860       870       880       890
125-94. seq   GTGACAGACCATCTACTCCTCTTATAGATCGTGGAATAGCATTGCACAAAATGATCAGGCTTATTACCAT
               TGACAGACCATCTAC CCTCT ATAGATCGTGGA TAGCATTGCACAAAATGATCAGGCTTATTACCAT
116. seq      TTGACAGACCATCTACCCCTCTCATAGATCGTGGAGTAGCATTGCACAAAATGATCAGGCTTATTACCAT
              1910      1920      1930      1940      1950      1960      1970
              900       910       920       930       940       950       960
125-94. seq   GGGCTTAGGCGGAGAAGGATATTTGAATTTTATGGGAAATGAATTTGGACATCCTGAGTGGATTGATTTT
              GGG TTAGGCGGAGAAGGATATTTGAATTTTATGGGAAATGAATTTGGACA CC GAGTGGATTGATTTT
116. seq      GGGATTAGGCGGAGAAGGATATTTGAATTTTATGGGAAATGAATTTGGACACCCCGAGTGGATTGATTTT
              1980      1990      2000      2010      2020      2030      2040
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Fig.5 (Cont).

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              970       980       990       1000      1010      1020      1030
125•94. seq  CCAAGAGGGGATCGACATCTGCCCAATGGTAAAGTAATTCCAGGGAACAACCACAGTTATGATAAATGCC
             CCAAGAGG GATC ACATCT CCCA TGGTAAA T  TTCC GGGAACAA  ACAGTTATGATAAATGCC
116. seq     CCAAGAGGTGATCTACATCTTCCCAGTGGTAAATTTGTTCCTGGGAACAATTACAGTTATGATAAATGCC
             2050      2060      2070      2080      2090      2100      2110
             1040      1050      1060      1070      1080      1090      1100
125•94. seq  GTCGTAGATTTGATCTAGGTGATGCAGACTATCTAAGATATCATGGAATGCAAGAGTTTGATCAGGCAAT
             G CGTAG TTTGATCTAGG  AT CA A  ATCT AGATATCATGGAATGCAAGAGTTTGATCA GCAAT
116. seq     GGCGTAGGTTTGATCTAGGCAATTCAAAGCATCTGAGATATCATGGAATGCAAGAGTTTGATCAAGCAAT
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             1110      1120      1130      1140      1150      1160      1170
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116. seq     TCAGCATCTTGAAGAAGCCTATGGTTTCATGACTTCTGAGCACCAATACATATCACGGAAGGATGAAAGG
             2190      2200      2210      2220      2230      2240      2250
             1180      1190      1200      1210      1220      1230      1240
125•94. seq  GATCGGATCATTGTCTTTGAGAGGGGAAACCTTGTTTTTGTATTCAACTTTCATTGGACTAACAGCTATT
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116. seq     GATCGGATCATTGTCTTCGAGAGGGGAAACCTCGTTTTTGTATTCAATTTTCATTGGACTAGCAGCTATT
             2260      2270      2280      2290      2300      2310      2320
             1250      1260      1270      1280      1290      1300      1310
125•94. seq  CAGATTACCGAGTTGGCTGCTTCAAGTCAGGAAAGTACAAGATTGTTTTGGACTCGGATGATGGCTTGTT
             C GATTACCGAGTTGGCTGCTT AAG CAGGAAAGTACAAGAT GT TTGGA TC GATGAT    TTGTT
116. seq     CGGATTACCGAGTTGGCTGCTTAAAGCCAGGAAAGTACAAGATAGTCTTGGATTCAGATGATCCTTTGTT
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             1320      1330      1340      1350      1360      1370      1380
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116. seq     TGGAGGCTTTGGCAGGCTTAGTCATGATGCAGAGCACTTCAGCTTTGAAGGGTGGTACGATAACCGGCCT
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             1390      1400      1410      1420      1430      1440      1450
125•94. seq  CGGTCCTTCATGGTATATGCACCATCTAGGACAGCAGTGGTCCATGCTTTAGTAGAAGATGAAG
             CG TCCTTCATGGT TA  CACCAT TAG ACAGCAGTGGTC ATGCTTTAGT GA GATGAAG
116. seq     CGATCCTTCATGGTGTACACACCATGTAGAACAGCAGTGGTCTATGCTTTAGTGGAGGATGAAG
             2470      2480      2490      2500      2510      2520      2530
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Fig.6.

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                 10        20        30        40        50        60        70
125-94. pro  SFGYHVTNFFAPSSRFGTPDDLKSLIDKAHELGLLVLMDIVHSHASNNTLDGLNMFDGTDSHYFHSGSRG
             SFGYHVTNF: A: SSRFGTPDDLKSLIDKAHELGLLVLMDIVHSHAS. NTLDGLNMFDGTD: HYFHSG: RG
116. pro     SFGYHVTNFYAASSRFGTPDDLKSLIDKAHELGLLVLMDIVHSHASTNTLDGLNMFDGTDGHYFHSGPRG
                 370       380       390       400       410       420       430
                 80        90        100       110       120       130       140
125-94. pro  HHWLWDSRLFNYGSWEVLRFLLSNARWWLEEYRFDGFRFDGVTSMMYTPHGLQVAFTGNYNEYFGYATDV
             HHW: WDSRLFNYGSWEVLRFLLSNARWWL: EY: FDGFRFDGVTSMMYT. HGLQV. FTGNYNEYFGYATDV
116. pro     HHWMWDSRLFNYGSWEVLRFLLSNARWWLDEYKFDGFRFDGVTSMMYTHHGLQVDFTGNYNEYFGYATDV
                 440       450       460       470       480       490       500
                 150       160       170       180       190       200       210
125-94. pro  DAVIYLMLVNDMIHGLFPEAVTIGEDVSGKPTFCIPVEDGGVGFDYRLHMAIADKWIEILKKRDEDWKMG
             DAV: YLML: NDMIHGLFPEAVTIGEDVSG. PT  CIPVEDGGVGFDYRLHMA: ADKW: EI: : KRDEDWKMG
116. pro     DAVVYLMLLNDMIHGLFPEAVTIGEDVSGMPTVCIPVEDGGVGFDYRLHMAVADKWVEIIDKRDEDWKMG
                 510       520       530       540       550       560       570
                 220       230       240       250       260       270       280
125-94. pro  DIVHTLTNRRWLEKCVAYAESHDQALVGDKTIAFWLMDKDMYDFMARDRPSTPLIDRGIALHKMIRLITM
             DIVH LTNRRWLEKCV: YAESHDQALVGDKTIAFWLMDKDMYDFMA DRPSTPLIDRG: ALHKMIRLITM
116. pro     DIVHMLTNRRWLEKCVSYAESHDQALVGDKTIAFWLMDKDMYDFMALDRPSTPLIDRGVALHKMIRLITM
                 580       590       600       610       620       630       640
                 290       300       310       320       330       340       350
125-94. pro  GLGGEGYLNFMGNEFGHPEWIDFPRGDRHLPNGKVIPGNNHSYDKCRRRFDLGDADYLRYHGMQEFDQAM
             GLGGEGYLNFMGNEFGHPEWIDFPRGD HLP: GK : PGNN. SYDKCRRRFDLG: : . . LRYHGMQEFDQA:
116. pro     GLGGEGYLNFMGNEFGHPEWIDFPRGDLHLPSGKFVPGNNYSYDKCRRRFDLGNSKHLRYHGMQEFDQAI
                 650       660       670       680       690       700       710
                 360       370       380       390       400       410       420
125-94. pro  QHLEEAYGFMTSEHQYISRKDEGDRIIVFERGNLVFVFNFHWTNSYSDYRVGCFKSGKYKIVLDSDDGLF
             QHLEEAYGFMTSEHQYISRKDE DRIIVFERGNLVFVFNFHWT: SYSDYRVGC: K: GKYKIVLDSDD LF
116. pro     QHLEEAYGFMTSEHQYISRKDERDRIIVFERGNLVFVFNFHWTSSYSDYRVGCLKPGKYKIVLDSDDPLF
                 720       730       740       750       760       770       780
                 430       440       450       460       470
125-94. pro  GGFNRLSHDAEHFTFDGWYDNRPRSFMVYAPSRTAVVHALVEDEENEAENEVES
             GGF. RLSHDAEHF: F: GWYDNRPRSFMVY: P. RTAVV. ALVEDE : : .  :  V. :
116. pro     GGFGRLSHDAEHFSFEGWYDNRPRSFMVYTPCRTAVVYALVEDEVENEVEPVAG
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Fig.7.

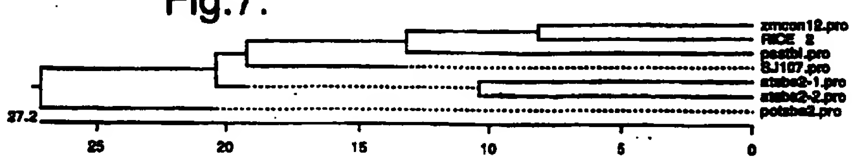
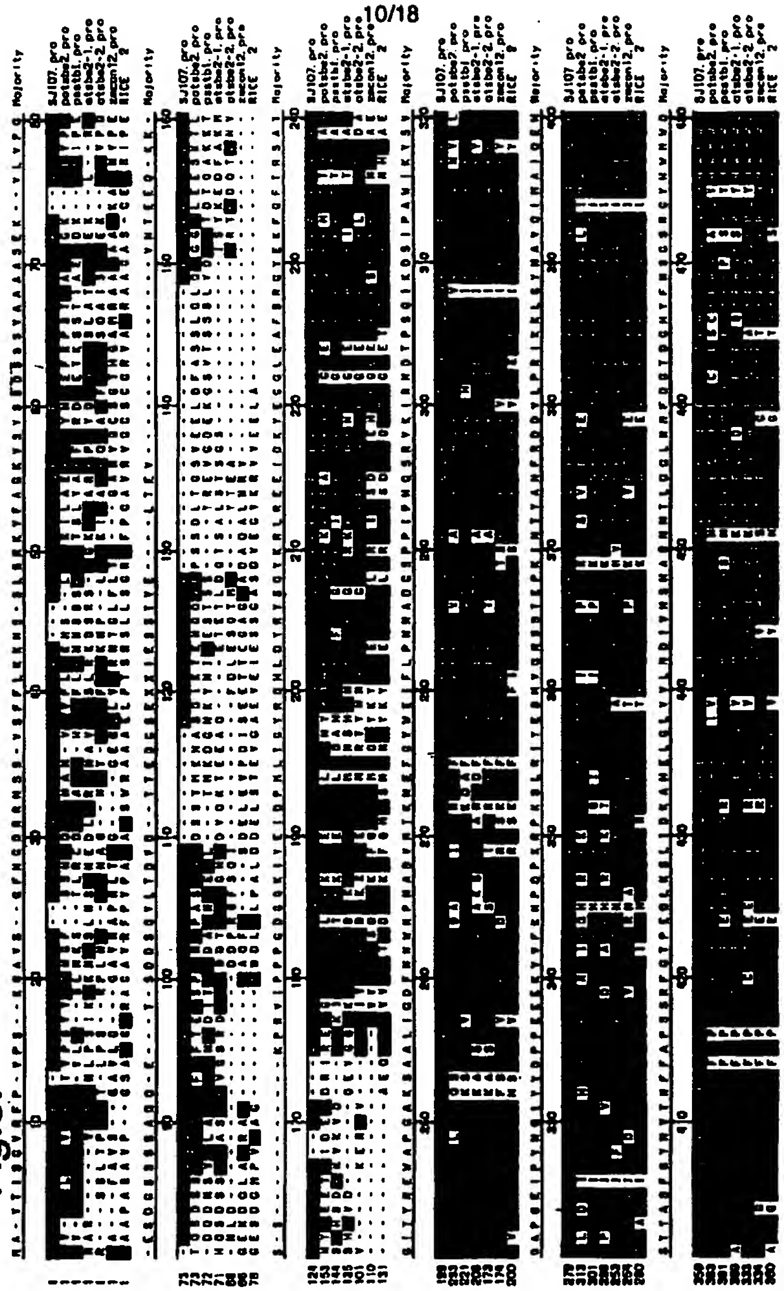


Fig.8.



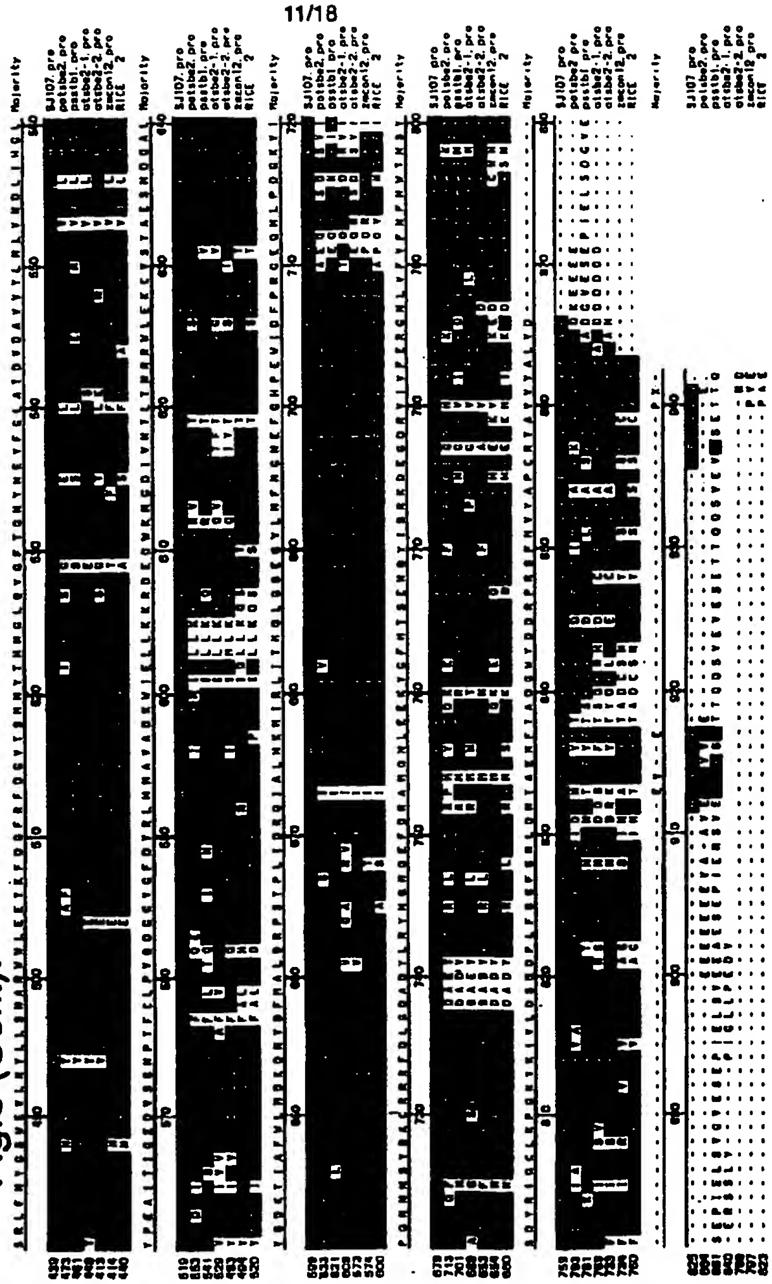


Fig.8 (Cont).

Fig.9.

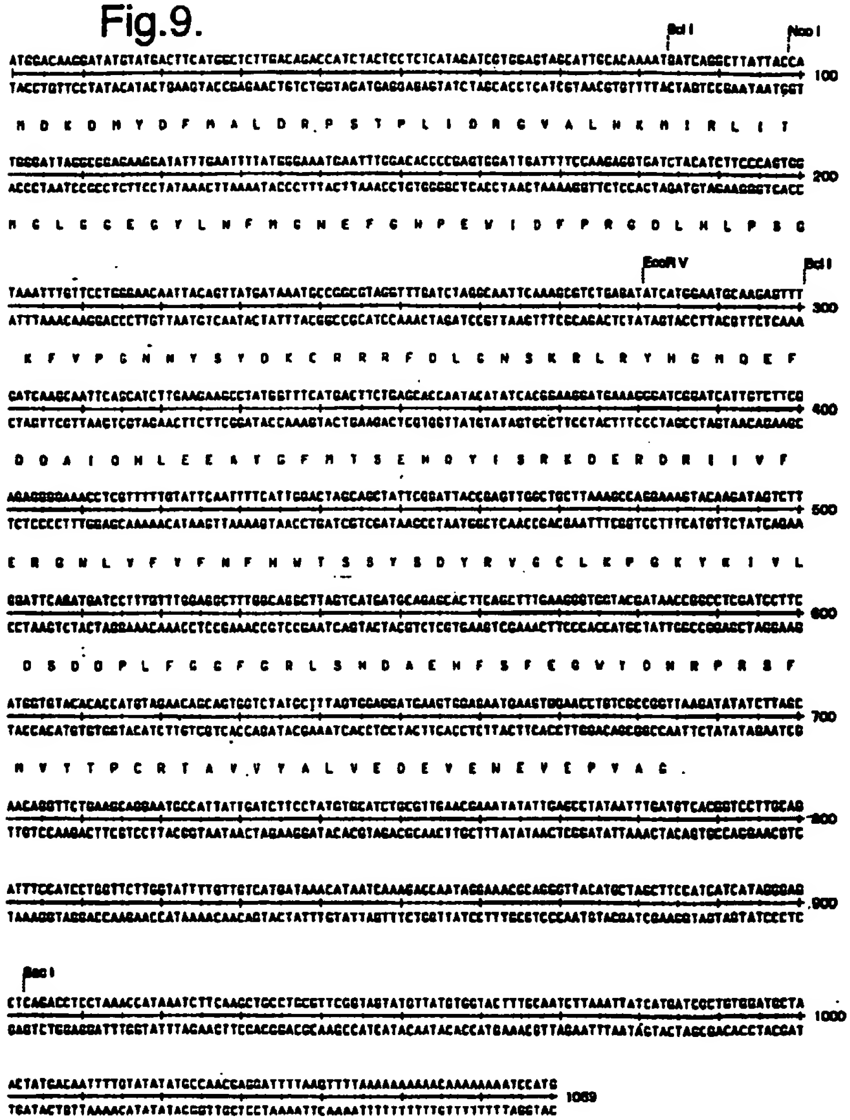

Bcl I
Nco I
100
200
EcoR V
Bcl I
300
400
500
600
700
800
900
1000
1069

Fig.10.

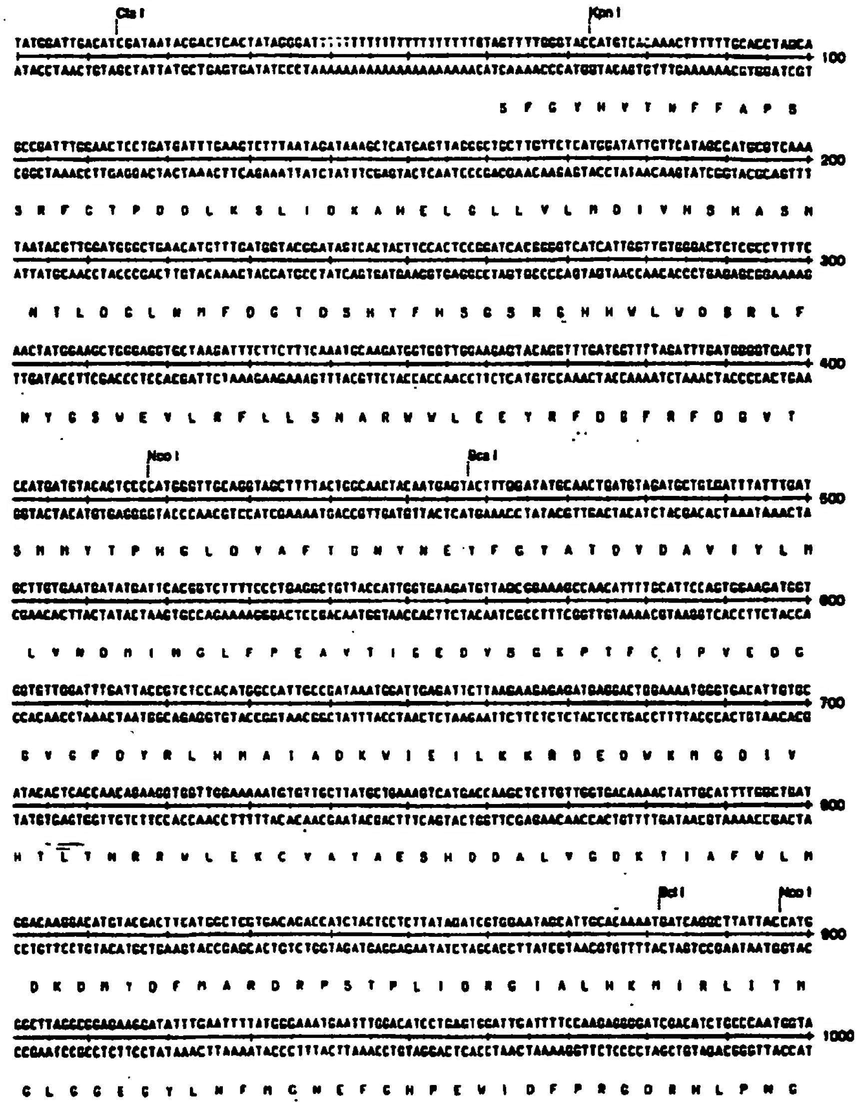

Cla I
Kpn I
Nco I
Sca I
Bcl I
Nco I
100
200
300
400
500
600
700
800
900
1000

Fig.10 (Cont).

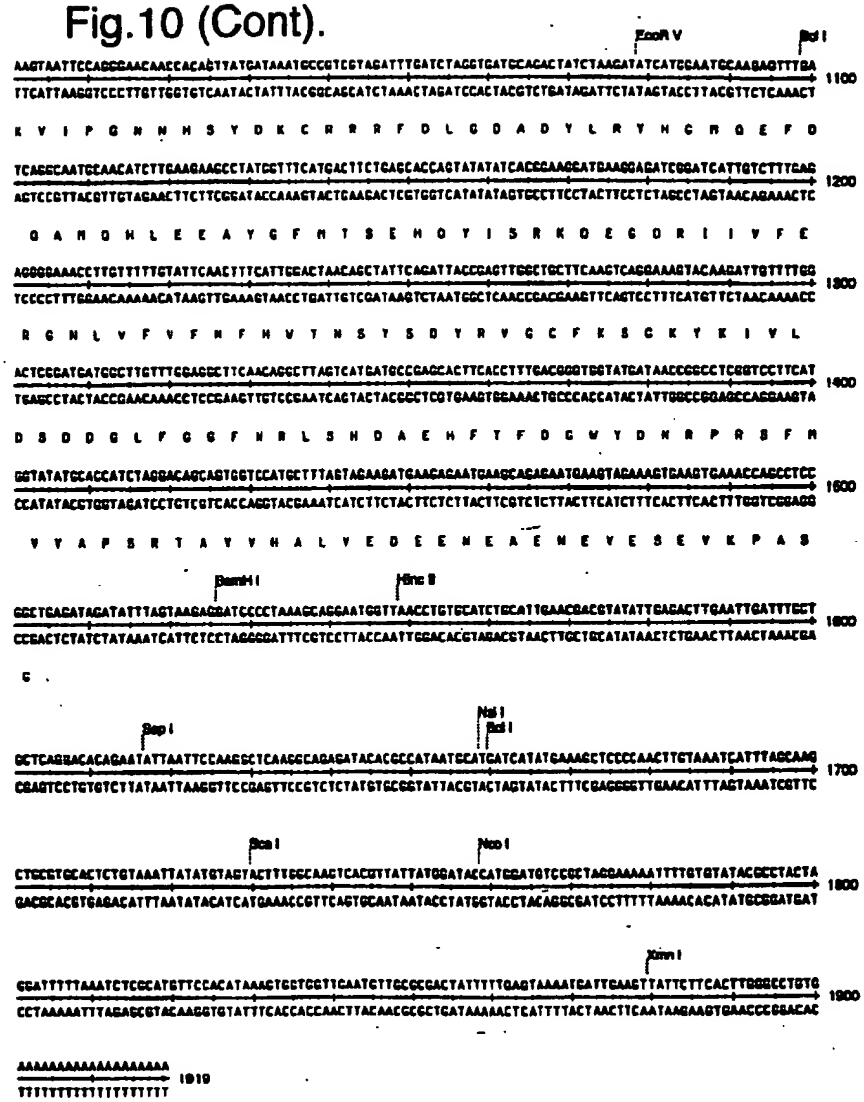
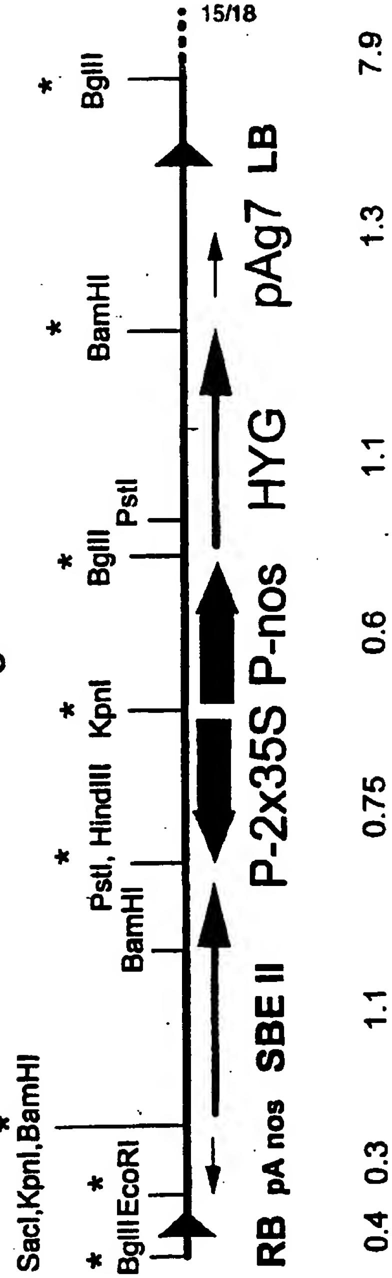

EcoR V
Bcl I
1100
1200
1300
1400
1500
BamH I
Hinc II
1600
Ssp I
Nsi I
Bcl I
1700
Sca I
Nco I
1800
Xmn I
1900
1919

Fig.11.


* BglII
* EcoRI
* SacI,KpnI,BamHI
BamHI
* PstI, HindIII
* KpnI
* BglII
PstI
* BamHI
* BglII
RB
pA nos
SBE II
P-2x35S
P-nos
HYG
pAg7
LB
0.4
0.3
1.1
0.75
0.6
1.1
1.3
7.9

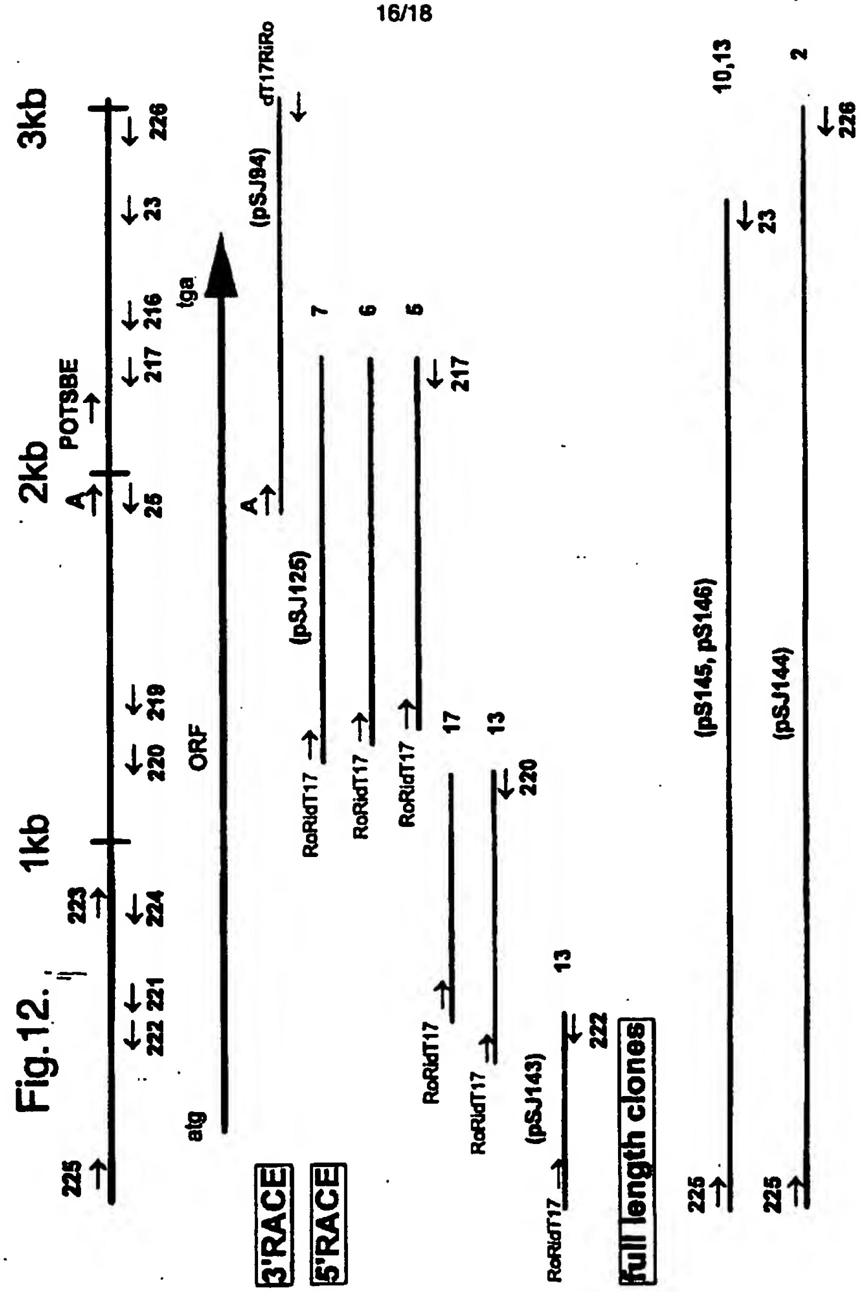

1kb
2kb
3kb
225
222 221
223
224
220
219
25
POTSBE
A
217 216
23
226
atg
ORF
tga
3'RACE
5'RACE
dT17RiRo
(pSJ94)
(pSJ125)
RoRidT17
7
6
5
217
17
13
220
(pSJ143)
222
full length clones
(pS145, pS146)
10,13
(pSJ144)
2

Fig.12.

Fig.13.

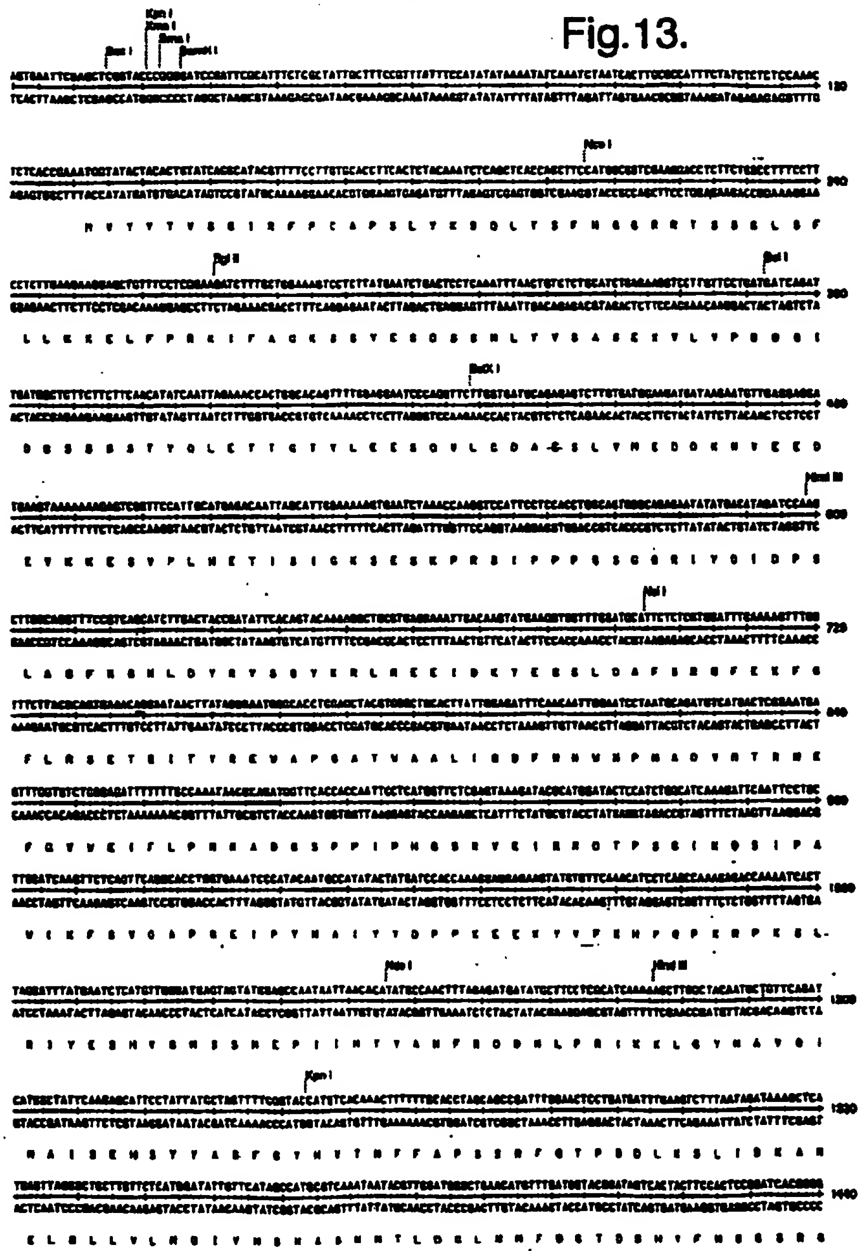


Fig.13 (Cont).

[illegible]